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INTRODUCTION

Among the fibre crops of India, Jute occupies a very prominent position both in its commercial and economic aspects. The commercial fibre is obtained chiefly from the bast of the Corchorus species, viz. *Corchorus capsularis* and *Corchorus olitorius*, both belonging to the family Tiliaceae. The Jute plants are extensively cultivated in Bengal and in the adjoining Provinces and form one of the chief economic crops of the Province of Bengal. Botanical investigations of these plants were chiefly confined in the past to their morphological aspects with particular reference to the anatomy of the fibre, etc. Very little work has so far been done on the cytology of these plants or that of the plants belonging to the family Tiliaceae.

The importance of the cytological study of the plants in general and of economic crops in particular is being increasingly recognized in recent years. A knowledge of the cytology and genetics of the various agricultural crops has been of untold benefit to the plant breeder in his efforts to improve the quality and yield of the various crops by hybridization. In this connection, the conception of the fundamental fact that chromosomes are the bearers of all the hereditary characters, made them the chief objects of intensive study, and a knowledge of their number, structure and behaviour through the various stages of mitosis and meiosis is highly essential for the correct understanding of the hereditary rôle they play from generation to generation in all living organisms. Further, a knowledge of the number of chromosomes and their behaviour in meiosis of the different varieties of an agricultural crop will give the clue to the possibilities of hybridization and breeding between the different varieties and species which may be evolved either naturally or as artificial mutation from existing types.

The literature on the cytology of plants belonging to the family Tiliaceae is very meagre as compared to other plant families of economic importance. Among the available literature on the subject, the work of Svensson-Stenar (1925) on *Tilia platyphyllos, Entelea Palmata* and *Sparmania africana* and of Wallisch (1930) on *Tilia platyphyllos, Tila cordata* and *Tilia argentea* may be mentioned. The haloid chromosome numbers for the plants listed above were, respectively, 40, 8, 80, 40, 36, and 40. Till very recently, the only cytological

work so far done on Jute was the ascertaining of the chromosome numbers of three species of Corchorus by Banerji (1932) and more recently, an abstract was published in the Proc. Ind. Sci. Congress by Nandi (1938) about a trisomic mutant among a Jute population. As the cytological details of the microsporogenesis of the Jute species have not been worked out by any of the above investigators, the present investigation of the same is undertaken with special reference to the structure of the early prophase chromosomes of meiosis and the results with the discussion of some of the fundamental problems of meiosis arising therefrom, form the subject of this paper. In passing, it may be mentioned here that an attempt is now being made by the author to produce artificial mutants in the Jute species by colchicine treatment.

MATERIAL AND METHODS

The material for this investigation was obtained from the crops grown on the grounds of the Bose Institute, Calcutta. Flower buds in various stages of development were collected and fixed on the spot at different hours of the day; best results were obtained from specimens fixed between 9 a.m. and 11 a.m. The root-tips were obtained by germinating the seeds in Petri dishes in the Laboratory.

The following fixing fluids were used for fixation :

(1) Medium Flemming.
(2) La Cour's 2BE.
(3) La Cour's 2BD.
(4) Navashin 1 and 2.

Of these, La Cour's 2BE and Navashin gave the most satisfactory results. An air-pump was used immediately after fixation to ensure thorough penetration of the fluid. The materials were kept in the fixing fluid for 24 hours and then washed, dehydrated in grades of alcohol, cleared in chloroform and embedded in paraffin in the usual way. Microtome sections were cut at thickness ranging from 8–15 μ and stained in Iodine-gentian-violet as per scheme given by La Cour (1931).

DEVELOPMENT OF THE MICROSPORES

Resting stage of the Microspore Mother-cells.

A cross section of one loculus of a young anther at the microspore mother-cell stage is shown in fig. 1. The pollen mother-cells in the resting condition are hexagonal in outline with no intercellular spaces between them. The tapetal cells are uninucleate and fairly regular in outline. The wall of the pollen mother-cell is very thin and the cell is filled with dense granular cytoplasm. In almost all the cells, the nucleus is central in position at this stage.

The nucleus of the resting pollen mother-cell is spherical in outline and occupies in most cases the centre of the cell. One mother-cell is shown enlarged in fig. 2. The nucleolus is very prominent in each cell and stains deeply with gentian violet. In almost all the nuclei, the nucleoli occupy an eccentric

position. The number of the nucleoli in each nucleus was mostly one, though in stray cases, two or three smaller nucleoli were noted. The chromatin threads at this stage are lightly stained and spread in the nuclear cavity in a loose manner (fig. 2). The loose chromatin threads lie over the nucleolus in some places simulating a possible connection with the latter.

Heterotypic Division.

Prophase.—With the commencement of the heterotypic prophase, the microspore mother-cells assume a more or less oblong or oval shape with rounded corners. At this stage, the nucleus is invariably located towards one side of the cell and the nucleus gradually increases in size. The nucleolus gradually begins to lose its chromaticity and the fine chromatin threads get thicker and shorter by longitudinal contraction. Consequently, the chromaticity of the threads increases and the individuality of the threads are fairly discernable at this stage. The structure of these early prophase threads (leptotene) is fairly clearly made out at this stage. They are seen to be double in constitution, each component being twisted about each other as shown in figs. 3 and 4, giving at some regions a beaded appearance according to the intensity of the stain in those regions. The structure of the chromatin threads at this stage has been variously interpreted by different investigators. The beaded appearance has been interpreted as chromomeres and the thread at leptotene stage has been regarded as single. In the material under investigation, these early prophase threads were examined very carefully with the controversial aspect of the question constantly in mind and it was observed that the threads were distinctly double and twisted about each other as shown in fig. 4, especially at the region marked A in the same figure. The same region is shown separately in fig. 6 where threads A and A′ represent how the dual twisted aspect of A will look like the beaded single aspect of A′ when stain is retained in the diamond areas formed by the twists of the dual threads. Fig. 5 is drawn from a cell that was a bit over-stained than the cell shown in fig. 4, and a careful examination showed that the early prophase threads of the over-stained cell presented a beaded and single appearance as shown in fig. 5. A portion of B of threads in fig. 5 where the threads show the beaded appearance clearly due to excess of stain is shown separately in fig. 6. The observations clearly show that the early prophase threads are double in constitution with no chromomeres, and the beaded appearance and the so-called 'chromomeres' often reported by some investigators are really misinterpretations of viewing a dual twisted thread when stain is retained in the diamond areas formed by the twists of the threads. The detailed discussion of this controversial point and its bearing on the theories of meiosis will be taken up later in the discussion part of the paper.

As the prophase advances, the chromatin threads which have shown their individuality even at the leptotene stage contract still further and the homologous pairs come together giving rise to the diplotene threads which lead to

the diakinesis stage. It is noteworthy to mention here that a stage like synizesis and the consequent knot formation of the chromatin threads was not observed in the present material.

Diakinesis.—The chromatin threads which have associated in pairs contract still further until each of the pairing homologues appears to be a thick mass with clear spaces here and there showing the duality of each of the pairing homologues (fig. 7). The pairing homologues show greater affinity at the ends in the initial stages of their association. In some cases only the ends get united to start with, and subsequently the homologues assume a parallel disposition. In other cases, both the ends of the homologues associate first and the middle regions remain apart giving a ring-like disposition to the pairing homologues. During this stage of diakinesis (fig. 7), the homologues bivalents move to the periphery of the nucleus, some of the bivalents even touching the nuclear membrane. At this stage, the nucleolus has practically lost most of its chromaticity and appears as lightly stained spherical outline towards one side of the nucleus (fig. 7).

The contraction of the pairing homologues proceeds still further and at late diakinesis (fig. 8) they become compact in form. During these contraction stages of the pairing homologues, they assume various configurations, sometimes one crossing over the other at the middle region so as to give the appearance of chismata. The number of bivalents could be very clearly counted at this stage and it was found to be 7 by counts of several diakinesis stages. The configurations of the seven bivalents as seen in late diakinesis are shown in fig. 8. By this time, the nucleolus has lost most of its chromaticity and shows signs of disintegration. The nuclear membrane also becomes fainter and gradually disintegrates as the metaphase commences.

Metaphase.—The metaphase stage of the heterotypic division is shown in fig. 9. The cell has by this time lost its hexagonal outline and becomes more or less spherical. The nuclear membrane and nucleolus have completely disappeared and the highly contracted metaphase bivalents are clearly seen in the centre of the cell. Several metaphase plates were counted and the number of bivalents was confirmed as 7, and the 7 bivalent chromosomes are shown in fig 9.

Even from the early metaphase, the bipolar nature of the spindle is evident and soon the bivalents arrange themselves at the equitorial region of the bipolar spindle as seen in fig. 10. The felt-like material of achromatic nature seen inside the nuclear cavity in the later stage of metaphase gradually converges to two points at the opposite ends of the cell giving rise to the bipolar spindle. Multipolar spindle commonly met with in most other plants was distinctly absent in the present material. The highly pointed nature of the heterotypic spindle is noteworthy as it is a condition necessitated by the absence of multipolar spindle.

Anaphase.—At the early anaphase, the bivalents which have come to the equitorial plate assume a dumbel-shaped configuration due to the pull exercised

by the fibres of the bipolar spindle (fig. 10). The components of the bivalents gradually separate and as the univalents are pulled to the poles, they become rounded in form as shown in fig. 11. As the univalents do not move simultaneously, slight lagging of the chromosomes are observed during the anaphasic separation. A mid-anaphase stage is shown in fig. 11. By the pull of the spindle-fibres, the univalents are drawn to the poles and a very late anaphase stage is shown in fig. 12. In both mid- and late-anaphase stages shown in figs. 11 and 12, the number of univalents could be counted as 7 at either poles, thus confirming the haploid number of chromosomes in Jute as 7.

Telophase.—The chromosomes aggregate together on reaching the poles; but at the same time they retain their identity. At the telophase, the reconstitution of the daughter nuclei takes place. As a first stage in this process, a hyaline zone is noted around the group of chromosomes at the poles so as to demarcate that zone from the rest of the cytoplasm. The highly contracted chromosomes of the metaphase and anaphase stages gradually begin to expand longitudinally, so much so the chromatin threads are visible now as intertwined structures. With the expansion of the chromosomes, they gradually lose their chromaticity and at this stage the nucleolus makes its appearance as a lightly stained spherical mass in each of the daughter nuclei. By this time the nuclear membrane is formed and the spindles gradually fade away and interkinesis follows. A telophase stage is shown in fig. 13.

Homeotypic Division and the formation of Microspores.

The process of homeotypic division follows the course of the usual mitotic division, the only difference being the suddenness of the whole process. Moreover, both simultaneous and successive divisions were noted in the present material. In the case of simultaneous division, the heterotypic anaphase chromosomes on reaching the poles get arranged themselves at the equitorial region of either poles and after the usual splitting of the chromosomes prior to the mitotic separation, move to the poles and the daughter chromosomes of the three of the developing microspores could be seen at three corners in one plane with the bipolar spindles connecting them as shown in fig. 14. Nuclear membrane is organized for each of the four microspore nucleus.

In the case of successive division, the process follows the usual mitotic cycle with interkinesis and the succeeding stages. The significance of both the types of homeotypic divisions will be discussed later. The chromosomes, on reaching the poles, gradually lose their identity and very soon the nuclear membrane is organized and the nucleoli make their appearance.

Cytokinesis.—The mode of cytokinesis in this material is by furrowing. The absence of a thickened plate at the equitorial region of the spindle denotes that cytokinesis here is by the furrowing and not by the cell-plate method. Constriction appears at the periphery of the cytoplasm and proceeds towards the centre, and along with it, a hyaline layer of the cytoplasm also moves

towards the centre. The furrows and the hyaline layer of protoplasm ulti-mately meet at the centre of the cell, thus dividing the general cytoplasm of the mother-cell into that of the four microspores. The cytoplasm of each microspore very soon secretes its own cell-wall and the four young microspores are seen in fig. 16. *The young microspores are spherical in outline and finally separate out from the pollen mother-cell wall by the gradual disintegration of the latter.*

Microspores.—As the microspore ripen to maturity, they either retain their spherical shape or in some cases assume an oblong shape so that both the types of microspores are met with in this material. The spiny outgrowths that are common on the microspore wall of most of the dicotyledonous plants are absent in the microspores of Jute. The oblong and spherical types of spores are shown respectively in figs. 17 and 18. The mature microspores show in most cases three germ pores as seen in fig. 18.

CHROMOSOME NUMBER IN MITOSIS AND MEIOSIS AND THEIR MORPHOLOGY

During the process of microsporogenesis described above, the chromosome number for Jute was ascertained at several stages. At meiosis, in several cases of diakinesis, seven pairing bivalents were clearly counted (figs. 7 and 8). At the heterotypic metaphase, seven bivalents were again counted in several plates (fig. 9). In the two stages of heterotypic anaphase shown in figs. 11 and 12, seven univalents were clearly counted at either poles in the same cells, thus confirming the haploid number of chromosomes in Jute as 7 and the diploid number as 14.

To obtain further confirmation of the diploid number, sections of the root-tips were stained and examined. Several mitotic metaphase plates were counted, all of which gave the diploid number of chromosomes in Jute as 14. It is noteworthy to mention here in this connection that direct evidence of the diploid number of chromosomes in Jute as 14 from mitotic division stages is here obtained for the first time. A camera lucida drawing of a somatic metaphase plate showing 14 chromosomes is shown below:—
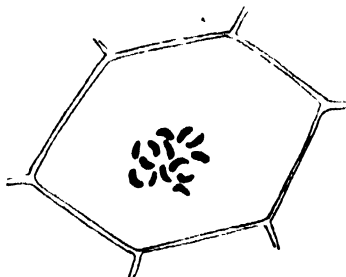
With regard to the size of the chromosomes at metaphase, no appreciable difference was noted between the different chromosome compliments. At

meiotic metaphase, the bivalents were highly contracted and were more or less spherical in shape as shown in fig. 9.   In the two species of Corchorus examined, viz. C. capsularis and C. olitorius, no difference in number or size of the chromosomes between the species was observed.

In the mitotic metaphase also, no difference in size was noted between the chromosomes compliments of a cell at metaphase; but in shape, the mitotic chromosomes were longer than broad and hence, they gave a rod-shaped appearance.   Owing to the small and highly contracted nature of the chromosomes at metaphase, the internal structural aspects of these chromosomes at this stage could not be clearly made out.

DISCUSSION

The structure of the early prophase threads (Leptotene) of Meiosis.—The structure of the early prophase threads of meiosis has been the subject of much controversy and discussion in recent years.   A correct understanding of the real structure of these threads is very vital in interpreting the theories of meiosis and also in explaining the constitution of the genes which are proved to be the bearers of all the heritable qualities.   Hence much care was devoted in the investigation of the present material by preparing several slides with different intensities of staining and examining them in detail with critical illumination so as to arrive at a correct understanding of the structure of these threads.

Before entering into the observational details of the present material with regard to the structural aspects of the leptotene threads, it is essential to point out how this question is related to such vital problems as the nature of the genes and the theory of meiosis.   Genes are supposed to be invisible ultimate units in the chromosomes carrying heritable qualities to the successive generations.   Aggregations of genes are supposed to give rise to visible units in the chromosomes called chromomeres.   Now, the chromomere theory postulates that the chromomeres are units present in the chromosome and clearly discernable as granules in the prophase stages of both mitosis and meiosis.   Many investigators have reported the presence of chromomeric granules in the prophase threads of both plants and animals.   Wenrich (1916) in Phrynotettix, Sands (1923) in Tradescantia, Belling (1928) in Liliaceous plants, Hedayetullah (1931) in Narcissus, Perry (1932) in Galanthes are a few among such investigators.   Another school of thought takes objection to the above view and holds the opinion that the so-called 'chromomeres' are not structural units of the chromosomes, but are mainly misinterpretations of the structural aspects of the prophase threads of both mitosis and meiosis.   As members of this school of thought, the names of Bolles Lee (1920), Smith (1932) in Galtonia, O'Mara (1933) in Lilium, Koshy (1933, 1934) in Allium, and (1937) in Aloe and Gregory (1935) in Carthamus, (1936) in Elettaria are a few that may be mentioned. These investigators deny the existence of the so-called 'chromomeres' as structural units in the prophase threads, but give several interpretations to the

chromomeric appearance of these threads. Thus, from a careful examination of the mitotic prophase threads of Carthamus and the meiotic prophase threads of Elettaria, the last-mentioned author has come to the conclusion that the threads are dual and twisted in constitution and the chromomeric appearance of these threads is due to the optical effect of viewing a highly stained twisted thread, especially when stain is retained in the diamond areas formed by the twists of these threads. Further, it was observed that a deeply stained preparation gave a chromomeric appearance; but when the same preparation was destained a little, the so-called chromomeric appearance vanished and the threads presented a double and inter-twined constitution.

To ascertain the real structural aspect of the leptotene threads with special reference to the points explained above, care was exercised in preparing lightly stained slides showing these stages and on critical examination of these slides it was found that no evidence of the actual existence of the so-called 'chromomeres' could be noted, but the threads presented a dual twisted aspect even from the early stages of the prophase as seen in figs. 3 and 4. In a highly stained cell shown in fig. 5, the same threads present a chromomeric appearance and hence it is clear that it is the intensity of the stain that makes the threads appear as chromomeric in constitution; but really the threads are double and twisted in structure with the absence of the so-called 'chromomeres'.

By the above observation and interpretation of the structural constitution of the early prophase threads of meiosis, the author for one, does not deny the existence of the genes or their rôle in heredity. It is not also denied that aggregations of these genes exist as visible units in the chromosomes which go by the name of chromomeres. But what is intended to be emphasized here is that the granular appearances noted in the prophase stages of both mitosis and meiosis cannot be interpreted as the actual visible aggregations of genes called chromomeres, because the true structural aspects of the leptotene threads demonstrated from this investigation as well as from those quoted above point to the conclusion that the so-called chromomeric granules are not real chromomeres, but only a faulty appearance and the consequent interpretation of viewing a highly stained inter-twined thread, especially when stain is retained in the diamond areas formed by the twists of the threads.

Thus, when the existence of the so-called 'chromomeres' is questioned, the gene conception also will have to be modified in the light of these observations. Investigations of the salivary gland chromosomes of Drosophila by Painter and Muller and few others have already thrown some light on the question of the visible units of the aggregations of genes in the chromosomes. The disc-like bands observed by them in the salivary gland chromosomes surely approach more closely to the visible aggregations of genes in the chromosomes than the faulty interpretation of these granular appearances of the prophase threads as the real chromomeres. It is up to further research on plants with smaller chromosomes also to find out the actual locations of the chromomeres (visible aggregations of genes) in such smaller chromosomes without being

satisfied with interpreting these granular appearances as the actual chromomeres.

The cause of pairing and disjunction in Meiosis.

The duality of the early prophase threads with the absence of the so-called 'chromomeres' demonstrated and discussed above has important bearing on some of the theories of the cause of pairing and disjunction of the homologous bivalents in meiosis. The cause of pairing of the homologous chromosomes is explained by one school of cytologists as being due to the attraction between pairs and repulsion between pairs of pairs, of the chromomeric threads. This theory evidently postulates that the early prophase threads of meiosis are single in constitution with chromomeres and the inherent tendency of the chromonematic threads to remain in pairs causes them to attract each other resulting in the pairing of the homologous chromosomes. But when split occurs in each of the pairing threads, pairs of pairs are in association which results in the repulsion between pairs of pairs and the consequent phenomena of disjunction in meiosis.

Though the above hypothesis is attractive in explaining the cause of pairing and disjunction in meiosis, it is not in agreement with structural details observed in chromosomes in the mitotic and meiotic cycles. In the mitotic cycle, the supporters of the above hypothesis assume singleness for the anaphase and telophase chromosomes. But the increasing evidence of anaphasic duality of mitotic chromosomes by investigators like Sharp (1929), Kaufmann (1926), Hedayetullah (1931), Perry (1932), Koshy (1933), Gregory (1935) and a number of other recent investigators cannot be denied. A quartipartite structure for the metaphase chromosomes of mitosis has been shown by most of the above investigators and recently Naithani (1937) has shown unmistakable evidence of a quartipartite structure in mitotic metaphase through actual photographs of the same in *Hyacinthus orientalis*. Such a quartipartite structure for metaphase necessarily gives rise to a dual structure for the anaphase and telophase chromosomes after the anaphasic separation and the observations of the investigators mentioned above also support the expectation. Now that the individuality of the chromosomes from one mitotic cycle to another is more or less an accepted fact, it cannot be expected that the telophase chromosomes will undergo any change in its fundamental structure during their passage from telophase to the prophase of the next division through the resting stage. So, the same dual structure should be expected for the prophase chromosomes whether it be in mitosis or meiosis, since the same pre-mitotic cell after somatic division functions as the meiotic cell and consequently no fundamental variation in structural details could be legitimately expected between the telophase and the succeeding prophase chromosomes. Direct evidence of leptotene duality has been given by recent investigators like Kaufmann (1932), Koshy (1934, 1937), Gregory (1936) and Banerji (1937).

The evidence from the present material also points unmistakably to the duality of the early prophase threads with the absence of the so-called 'chromomeres'. Thus when duality for the early prophase threads is established both by direct evidence from the present material as well as by the structure noted for chromosomes through the stages leading to it, the hypothesis explaining the cause of synapsis (pairing) between homologues chromosomes explained before is untenable as it rests on the fundamental conception that early prophase threads in meiosis are single and that pairing is brought about by the attraction between pairs. On the same basis, the cause of disjunction in meiosis, viz. due to repulsion between pairs of pairs, is again untenable as the pairing elements are already double in constitution and no subsequent split is initiated in them through the stages of meiotic prophase.

Thus when the cause of pairing (synapsis) and the separation (disjunction) between homologous chromosomes in meiosis as enunciated by the above hypothesis is explained as untenable in the light of the observations of the real structure of leptotene threads, the cause for the same has yet to be found out by further investigation. In the opinion of the author, synapsis is brought about by the attraction of the like genes or their visible aggregations situated in the parental chromosomes and disjunction occurs when the necessary interchange of energies or qualities has been effected by the pairing genes. This view of the cause of pairing, viz. like gene pairing with like gene, finds able support from Muller (1937) in one of his recent papers on the subject in which he explains it with the general statement 'specific autoattraction of like with like'. This view has been further amplified by Snell (1938) in his consideration concerning the structure of chromosomes and genes. Muller postulates a specificity for this gene attraction and further he states that this attraction is capable of acting even at distances. In the opinion of the author, this specificity of attraction of the homologous genes may account for the different configurations of the pairing threads. As the particular genes with specific attraction may be situated at distances when the pairing threads come together, loops, inversions, translocations, etc., have to be formed for the specific genes to be brought together for the exchange of qualities. Since the chromosomes are the carriers of genes and their visible aggregations, the sum total of the attractions of the specific homologous genes of the pairing threads will bring them together which will result in synapsis. When the forces of attraction between the homologous genes are exchanged either as energy transmissions, or through chiasmata or even by segmental interchange, the pairing homologues will separate thus resulting in the disjunction of the paired threads in meiosis.

Mode of pairing in Meiosis.

The mode of pairing of the homologues chromosomes has been a topic of great controversy in the cytological world. Telo- (end to end) and Para- (side by side) synapsis have been the two main modes of pairing advocated by schools of investigators. It is needless here to go into the historical retrospect

of the whole problem as it has already appeared in several treatises and papers on the subject. Suffice it to quote here in this connection Sharp's passage in his book (1934) dealing with this controversy. 'For many years cytology witnessed a battle between " parasynapsis" (lateral synapsis) and " telosynapsis" (synapsis primarily end to end), but the battle has now practically ended with the retirement of telosynapsis from its last major stronghold Oenothera.' In spite of this summing up by Sharp on this controversy in favour of para-synapsis, evidences are forthcoming in several subsequent papers that the two modes of pairing are evidently two stages in the pairing of the same homologues according to the location and intensity of the units of attraction in the homolo-gous pairs. If the force of attraction of the genes at the ends of the pairing homologues is greater, the threads join by their ends which will give a telo-synaptic appearance to start with. Subsequently, according to the degree of attraction between the homologous genes in the middle regions of the pairing threads, the latter assume a parallel disposition giving a parasynaptic appearance to the mode of pairing. The problem was examined in detail in several stages of early and late diakinesis in the present material. In most cases, in the early diakinesis stage (fig. 7), the attraction between the pairing threads was more at the ends, so much so, the pairing bivalents gave a more or less ring-like appearance at this stage. But in later stages of diakinesis (fig. 8), it was noted that the highly contracted homologous pairs assumed a parallel disposition in pairing and in one bivalent, a chiasmata is clearly seen at the middle region, the ends remaining separate. From these observations, it is clear that the two modes of pairing, viz. telo- and parasynapsis are essentially two stages in the process of pairing, the sequence of the parallel or end to end association depending mainly upon the location of the homologous genes in the pairing threads which have the maximum attraction. If the force of attraction of the homologous genes at the ends of the threads are greater than that of the genes in the middle region, an end to end association is initiated as seen in most of the bivalents of the present material. But if the force of attraction of the homologous genes at one end of either threads is greater than that at the other ends, only the first two ends associate first, and subsequently, by the movements of the threads in the nuclear cavity, a parallel disposition is brought about for the pairing threads, the other ends may or may not join according to the forces of attraction at those ends. One bivalent at such a mode of pairing is seen in both the early diakinesis and late diakinesis stages shown in figs. 7 and 8.

The mode of Homeotypic Division and Cytokinesis.

Two types of homeotypic divisions, viz. successive and simultaneous, are generally met with in plants, the former mostly in Monocotyledons and the latter mostly in Dicotyledons. In successive division, the chromosomes of the

meiotic anaphase complete the later stages of the first division, namely telophase and interkinesis before the homeotypic division is initiated in the usual mitotic way.   Here, one division follows another in quick succession.   In a simultaneous division, most of the later stages of the first division and the prophase of the second division are eliminated.   Here the heterotypic anaphase chromosomes simply arrange themselves at the equitorial region, split and separate forming the four anaphase groups which subsequently through telophase form the microspores.

In the present material, the mode of homeotypic division was observed to take place in both ways, some mother-cells taking up the successive type while others the simultaneous type of division.   Fig. 13 shows the clear telophase of the first division indicating that a successive mode of division is going to take place for that mother-cell, while fig. 14 shows three anaphase groups after the second division suggesting the simultaneous type of division for that cell.   Generally, in one plant species, only one type of division occurs, but this material is interesting in that both the types of divisions are present in the same plant species.

Coming to the problem of cytokinesis, two methods are commonly met with in this process, viz. by furrowing and by the cell-plate method.   Generally cleavage by furrowing is characteristic of higher animals and cytokinesis by cell-plate method is common in plants.   The furrowing method, though commonly met with in animals, is also observed by Farr (1922), Castetter (1926), Gates (1927), Passmore (1930) and Asana and Sutaria (1932) in the respective plants of their investigation.   Cell-plate method of cytokinesis has been reported in plants and its process explained by Robyns (1929), Belar (1929), Becker (1932) and Gregory (1936).

The mode of cytokinesis in the present material is by the furrowing process.   The absence of a thickened region at the equitorial region of the spindle in fig. 13, denotes even at the very outset that the mode of cytokinesis here is not by the cell-plate method.   The daughter nuclei of the homeotypic division are first surrounded by a hyaline layer.   The periphery of the general cytoplasm of the mother-cell progressively constricts inwards until the cytoplasmic furrows formed by the constriction meet in the centre of the cell separating the four pollen grains (microspores).   The cytoplasm of the four developing microspores soon secretes walls of its own and thus the development of the microspores is completed.

The individual microspores later emerge from the mother-cell and ripen to maturity.   The pollen grains are spherical and in some cases oblong in shape and are devoid of spiny outgrowths.   Generally spines are characteristic of the microspores of most dicotyledons and are absent in most monocotyledons. This material is interesting in that the spiny outgrowths generally characteristic of the group are completely absent here.

SUMMARY AND CONCLUSIONS

The microsporogenesis of Jute is investigated in detail and the cytological details of the whole process through the stages of meiosis has yielded the conclusions summarized below:—

The haploid number of chromosomes for two species of Corchorus, viz. C. capsularis and C. olitorius, is ascertained to be seven from several counts of the heterotypic metaphase and anaphase stages. The diploid number of chromosomes for the above species is also ascertained to be 14 from several of the mitotic metaphase plates from the root-tips. No difference in size or number was noted between the two species.

The stages of meiotic division leading to microsporogenesis were worked out in detail in C. capsularis with special reference to certain controversial points relating to the structure of chromosomes and the theories of meiosis, such as the cause and mode of synapsis and disjunction, mode of cytokinesis, etc.

With regard to the structural aspect, the constitution of the early prophase chromosomes was examined in detail and it is found that they are chromonematic in constitution with a dual twisted aspect with the absence of the so-called ' chromomeres '.

In this connection, the chromomeric hypothesis of chromosome structure is discussed and the so-called 'chromomeres' are shown to be no structural units of the early prophase threads, but are merely the optical effect of viewing a highly stained and dual twisted threads especially when stain is retained in the diamond areas formed by the twists of the chromonematic threads.

The causes of meiotic pairing and disjunction are discussed in detail. The hypothesis that the cause of pairing and disjunction is due to the attraction between pairs and the repulsion between pairs of pairs is shown to be untenable in the light of the duality of the early prophase threads demonstrated in the present material. But it is concluded that the cause of meiotic pairing is due to specific attraction between homologous genes in the pairing bivalents, and disjunction occurs when the paired genes have exchanged the requisite energies or even qualities either by mutual association, by chiasmata or by segmental interchange as the case may be.

The mode of pairing in meiosis is then discussed in detail and it is concluded from observations in the present material that telo- and parasynapsis are different stages in the mode of pairing according to the intensity of attraction between the various genes present in different regions of the pairing bivalents. In the present material, pairing is initiated in most bivalents by an end to end association which later on assumes a parallel disposition towards the later stages of pairing.

Simultaneous and successive types of homeotypic divisions are found to occur in the present material in the formation of the microspores.

The mode of cytokinesis in tetrad formation is by the furrowing process.

The microspores are spherical and in some cases oblong in shape and are devoid of any spiny outgrowths which are characteristic of the microspores of most other dicotyledons.

In conclusion, I wish to express my sincere thanks to Dr. D. M. Bose, Director of the Institute, for providing me with the facilities in connection with the preparation of this paper.

LITERATURE CITED

Asana, J. J. and Sutaria, R. N.—Ind. Jour. Bot. Sci., (1932), II, 181–87.

Banerji, I. and Das, M. C.—'The development of the microspores in *Trichosanthes dioica*.' Ind. Jour. Agri. Science, (1937), 7, 497–510.

Banerji, I.—'Chromosome numbers of Indian Crop Plants.' Ind. Jour. Bot. Sci., (1932), 2, p. 82.

Becker, W. A.—'Protoplasma.' Acta. Soc. Bot. Polon., (1932), 9, 351–419.

Belar, K.—'Mechanism of Mitosis.' Zeit. Zellf. Mikr. Anat., (1929), 10, 73–134.

Bolles Lee, A.—'The structure of certain Chromosomes and the mechanism of their division.' Quart. Jour. Micro. Sci., (1920), 65, 1–32.

Castetter, E. F.—'Cytological studies in Cucurbitaceae.' Amer. Jour. Bot., (1926), 12, 270–86.

Farr, C. H.—'Meiotic Cytokinesis in *Nelumbo*.' Amer. Jour. Bot., (1922), 9, 286–306.

Gates, R. R.—'Pollen tetrad formation in *Lathraea*,' 1927. La Cellule, 35, 49–58.

Gates, R. R.—'Chromosomes structure in *Trillium*.' Nature, (1937).

Gregory, P. J.—'Cytological studies in Safflower.' Proc Ind. Acad. Sci., (1935), II, 763–777

Gregory, P. J.—'The Floral Morphology and Cytology of *Elettaria Cardamomum*.' Jour. Linn. Society, London, (1936), 50, 363–391.

Hedayetullah, S.—'On the structure and division of the Somatic Chromosomes in *Narcissus*.' Jour. Roy. Micro. Soc., (1931), 51, 347–86.

Kaufmann, B. P.—'Chromonematic structure and its relation to Chromosomes cycle.' Amer. Jour. Bot., (1926), 13, 59–80.

Kaufmann, B. P.—'Chromonemata in somatic and meiotic mitosis.' Amer. Nat., (1931), 65, 555–58.

Koshy, T. K.—'Chromosomes studies in *Allium* I.' Jour. Roy. Micro. Soc., (1933), 53, 299–318.

Koshy, T. K.—'Chromosome studies in *Allium* II.' *Ibid.*, (1934), 54, 104–120.

Koshy, T. K.—'Number and behaviour of the Chromosomes in *Aloe litoralis*.' Ann. Bot., New Ser., (1937), I, 43–58.

La Cour, L.—'Improvements in everyday technique in Plant Cytology.' Jour. Roy. Micro. Soc., (1931), 51, 119–26.

Muller, H. J.—Scientific Monthly, (1937), 44, 210–214.

Naithani, S. P.—'Chromosome studies in *Hyacinthus orientalis*.' Ann. Bot., (1937), I, 129–46.

Nandi, H. K.—'Cytogenetical studies on Jute.' Abstract in the Proc. Ind. Sci. Congress, (1938), 142-43.

O'Mara.—'Division of the generative nucleus in the Pollen tube of *Lilium*.' Bot. Gaz., (1933), 94, 567–78.

Passmore, S. F.—Bot. Gaz., (1930), 90, 213–23.

Perry, K. M.—'Mitosis in *Galanthus nivalis*.' Jour. Roy. Micro. Soc., (1932), 52, 344–56.

Robyns, W.—'La Figure achromatique, etc.' La Cellule, (1929), 39, 85–119.

Sands, H. C.—'The structure of the Chromosomes in *Tradescantia virginica*.' Amer. Jour. Bot., (1923), 10, 343–60.

Sharp, L. W.—'Introduction to Cytology.' (1934), McGraw Hill, p. 337.

Sharp, L. W.—'Structure of large somatic Chromosomes.' Bot. Gaz., (1929), 88, 349–82.

Smith, F. H.—'The structure of the Somatic and Meiotic Chromosomes of *Galtonia candicans.*' La Cellule, (1932), 41, 243–64.

Snell, G. D.—'Some considerations concerning the structure of·chromosomes and the genes.' Amer. Nat., (1938), 72, 84–94.

Svensson-Stenar, A. H.—'Embryologische Studien I.' Acad. Abhand Uppsala, (1925), 1–195.

Wallisch, R.—'The Chromosomen verhaltnisse bei *Tilia platyphyllos, Tilia cordata* und *Tilia argentea.*' Oesterr. Bot. Zeit., (1930), 79, 97–106.

Wenrich, D. H.—'The spermatogenesis of *Phrynotettelx magnus* with special reference to Synapsis and the Individuality of the Chromosomes.' Bull. Mus. Comp. Zool. Harward Coll., 60, 57–133.

EXPLANATION OF FIGURES

The figures represent cytological details of the various stages of the microsporogenesis of Jute and were drawn at table level with the aid of a camera lucida. Special care was taken to obtain critical illumination with the aid of colour filters and ponito-light. The magnification was as indicated for each.

FIG. 1.   One loculus of young anther showing the closely packed pollen mother-cells. Note their distinct hexagonal shape.   × 400.

FIG. 2.   A single resting pollen mother-cell enlarged to show its contents. Note the big size and the high chromaticity of the nucleolus. The chromatin threads are very thin and less chromatic at this stage.   × 1600.

FIG. 3.   A cell showing the early prophase stage. Note the duality of the early prophase threads. The nucleolus has become smaller and less chromatic than the previous stage.   × 1600.

FIG. 4.   Another cell showing early prophase threads. This cell is less stained and shows the duality of the leptotene threads, especially at the region marked A.   × 1600.

FIG. 5.   A highly stained cell showing the early prophase threads. Note how in most regions of the threads, especially at B, the threads present a chromomeric and single appearance.   × 1600.

FIG. 6.   Threads marked A and B in this figure are portions of the threads marked similarly in figs. 4 and 5, respectively, and are shown separately to indicate how the less stained thread A has dual twisted aspect while the highly stained thread B has a single and chromomeric appearance. Thread A′ represent how the same dual and twisted thread A will present a chromomeric appearance when excess of stain is retained in the diamond areas formed by the twists of the threads.   × 1600.

FIG. 7.   Early diakinesis stage showing the mode of pairing of the seven bivalents. Note the ring-like disposition of most of the bivalents due to their ends coming into association first.   × 1600.

FIG. 8.   Late diakinesis stage showing the subsequent parallel disposition of most of the pairing threads (bivalents). The pairing threads that had moved to the periphery in the early stage shown in fig. 7, are coming more and more to the centre at the late diakinesis stage. Note the gradual disintegration of the nucleolus.   × 1600.

FIG. 9.   Metaphase stage showing the 7 bivalents in the centre of the cell. The nuclear membrane and the nucleolus have completely disappeared now.   × 1600.

FIG. 10.   Very early anaphase stage of the first division showing the highly pointed and distinctly bipolar spindle.   × 1600.

FIG. 11.   A mid-anaphase of the first division showing the 7 univalents passing to either poles, thus fixing the diploid number of chromosomes as 14.   × 1600.

FIG. 12.   A late anaphase of the first division showing again the seven univalents at either poles.   × 1600.

FIG. 13. Telophase of first division. Note the formation of the nuclear membrane for each of the daughter nuclei. The nucleolus has also begun to appear in each. Note the absence of a thickened region at the equitorial region of the spindle indicating that cytokinesis here is not by the cell-plate method. × 1000.

FIG. 14. Anaphase of the homeotypic division showing chromosomes of the three daughter nuclei indicating that a simultaneous type of division has taken place in that cell. × 1000.

FIG. 15. Three young microspores seen in one plane in the mother-cell wall. × 600.

FIG. 16. The tetrads within the mother-cell wall. Here the four young microspores are seen in one plane. × 600.

FIG. 17. The mature microspore (pollen-grain) which is oblong in shape and devoid of spines. × 1000.

FIG. 18. The mature microspore which is spherical in shape and again devoid of spines. Note the three germ-pores on the wall of the microspores. × 1000.

The following figures (figs. 19–27) are microphotographs of some of the stages of Microsporogenesis shown in figs. 1–18 :—

FIG. 19. Prophase threads of meiosis showing no chromeric appearance. The actual dual constitution of the threads is not very clear in the photo as its dual structure could not be brought out well in photographic focus.

FIG. 20. Early prophase threads of meiosis showing a chromomeric appearance at some regions owing to excess of stain being retained in the diamond areas formed by the twists of the threads.

FIG. 21. Heterotypic metaphase stage showing seven bivalents.

FIG. 22. Anaphase stage of heterotypic division showing the univalents moving to either poles.

FIG. 23. Telophase stage of heterotypic division showing the reconstitution of the daughter nuclei at either poles.

FIG. 24. Late anaphase of the homeotypic division showing three sets of the daughter chromosomes of the tetrad.

FIG. 25. Tetrad stage of the microsporogenesis showing three young microspores in the mother-cell wall.

FIG. 26. Microspores which are oblong in shape.

FIG. 27. Spherical microspores with three germ-pores and with the spore wall devoid of spines.
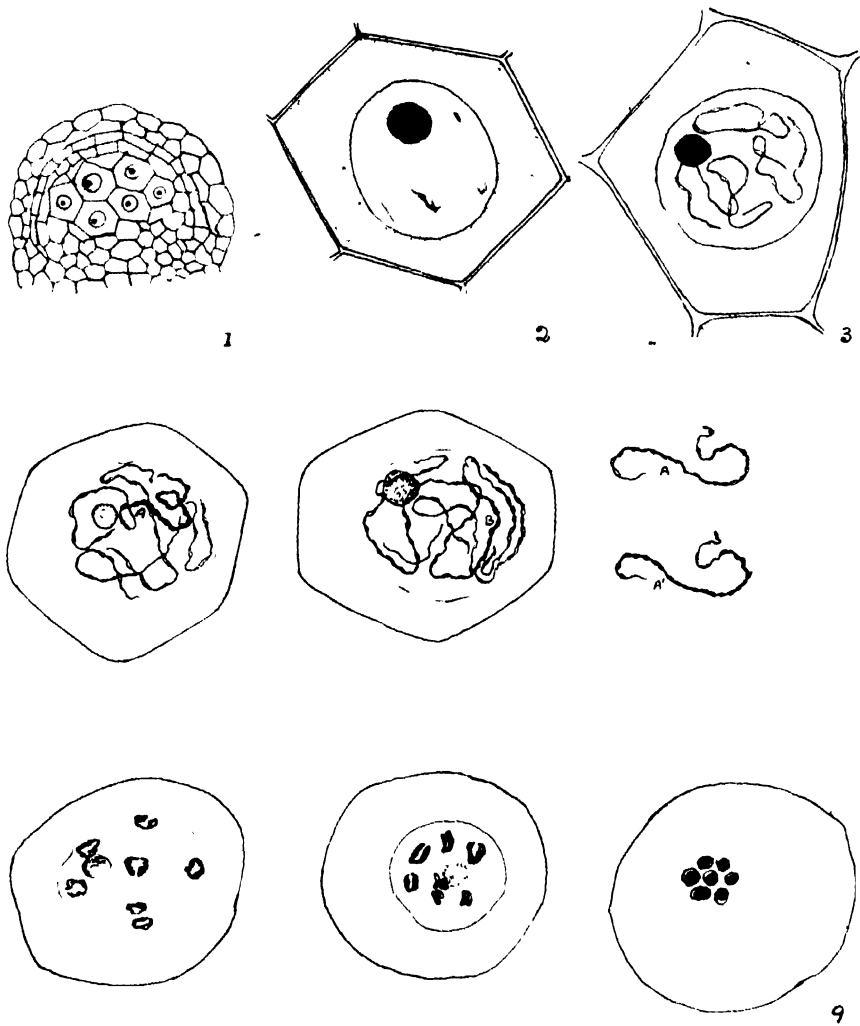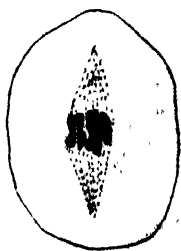
10   *11*   *12*
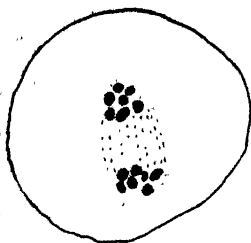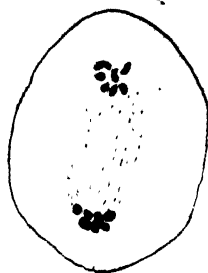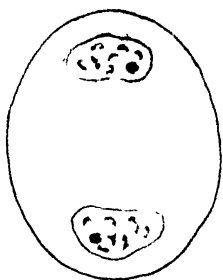
13   *14*   *15*
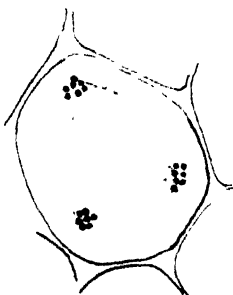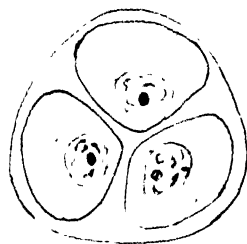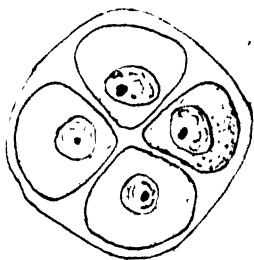
16   *17*   *18.*
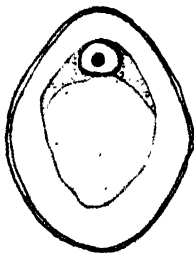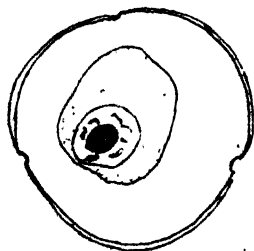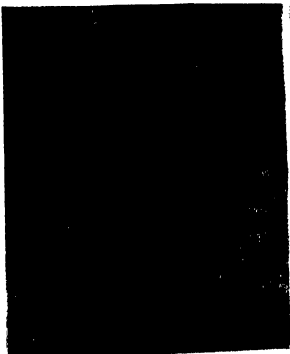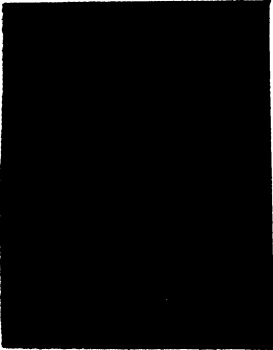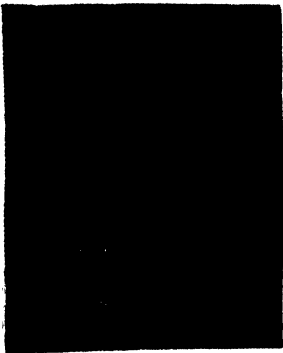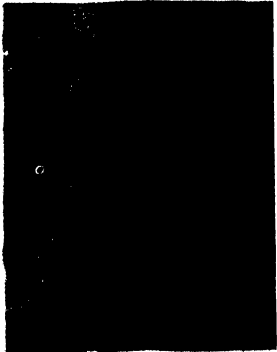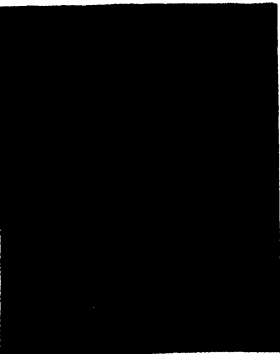
II. THE THEORY OF CONDUCTION IN THE SOLAR ATMOSPHERE *

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SUMMARY

The motion of electrons in an ionized gas under electric and magnetic fields is investigated from the Kinetic theory of gases based on quantum mechanics. In the first three articles the solution of Boltzmann's equation, expression for current and the time of relaxation are deduced, and in the last two articles the formulae for conduction are obtained and the results are discussed in reference to the solar atmosphere.

INTRODUCTION

The phenomenon of conduction in solar atmosphere has got great significance in connection with the behaviour of sun's general magnetic field in the reversing layer. It was established by Hale in 1913 that the sun possesses a general magnetic field much like that of the earth, the intensity of which decreases rapidly outwards in the radial direction, falling from 150 Gauss to an amount of 30 Gauss † within a height of about 300 km. only. Different theories, namely the drift current, diamagnetic and the dynamo theories have been put forward by Chapman,[1] Gunn[2] and Ferraro[3] to explain this radial limitation of the solar magnetic field. It has been felt that for a quantitative study of this phenomenon it is of foremost importance to know exactly the motion of the electrons, i.e. the conduction currents under the circumstances prevailing in the solar atmosphere. The systematic investigation of this problem was first initiated by Chapman,[4] but he himself realized, as mentioned in an addendum to his paper, the uncertainties of large amount in his own estimation of the value of the mean free path which is involved in the formulae for conduction. This was, therefore, subject to careful revision by Ferraro[5] and more elaborately by Cowling[6] from a general treatment of the Kinetic theory of gases, the results of their calculations being so remarkably different that Chapman's main conclusion was completely reversed. But unfortunately these treatments were also not free from defects. As for example, by compensating the Hall current by the application of an external field in its opposite direction Cowling found that there was no change in conduction due to the

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* The paper forms a part of the thesis submitted in November, 1937, for the Premchand Roychand Studentship of the Calcutta University. The publication has been delayed due to some unavoidable circumstances.

† These values are adopted according to Rosseland's correction of the original values which were 50 and 10 Gauss respectively. S. Rosseland, Theoretical Astrophysics, 1936, p. 219.

transverse magnetic field—a conclusion which is obviously not correct. He himself admits that 'this result is not absolutely exact; an exact calculation would show that the magnetic field does produce some alteration'. In view of these difficulties associated with such an important problem we shall attempt in this paper to obtain an exact expression for conductivity from a more general and rational treatment of the Kinetic theory of gases founded on Quantum mechanics. The application of these results to the problem of the radial limitation of the solar magnetic field will be communicated in a subsequent paper. In the first three articles of this paper the solution of the Boltzmann's equation, the expressions for the current and the time of relaxation are deduced. In the fourth article the formulae for conduction and their quantitative evaluation for the solar atmosphere are worked out. The conductivity along the electric field when the Hall current is prevented is discussed in the last article.

§1. The electrons of the solar atmosphere are subject to a magnetic field due to the sun which, as shown by Panekoek, is also charged up to a certain electric potential. The problem of conduction in solar atmosphere reduces, therefore, to the investigation of the motion of electrons in electric and magnetic fields, account being taken of the collisions between the electrons and the ions. If we denote the distribution function of the electrons by f, then the change in f due to the combined effect of the electric and magnetic fields on the one hand and the collision between the electrons and the ions on the other hand is governed by the well-known Maxwell-Boltzmann's equation and is given by

$$\frac{\partial f}{\partial t}+\left(\frac{\partial f}{\partial t}\right)_{\text{electric field}}+\left(\frac{\partial f}{\partial t}\right)_{\text{magnetic field}}=\left(\frac{\partial f}{\partial t}\right)_{\text{collisions}} \qquad .. \quad (1)$$

or, writing explicitly we obtain, for the stationary case,

$$\frac{h}{m}\left(\vec{k}\ \text{grad}_{\vec{r}}f\right)+\frac{1}{h}\left(\vec{F}\ \text{grad}_{\vec{k}}f\right)$$

$$=\int\int\left[\ \text{W}(\vec{k'}\,\vec{k}\,\vec{l'}\,\vec{l})f_{\vec{k'}}\left(1-f_{\vec{k}}\right)f_{\vec{l'}}\left(1-f_{\vec{l}}\right)\right.$$

$$\left.-\text{W}(\vec{k}\,\vec{k'}\,\vec{l}\,\vec{l'})f_{\vec{k}}\left(1-f_{\vec{k'}}\right)f_{\vec{l}}\left(1-f_{\vec{l'}}\right)d\Phi_{\vec{k'}}\,d\Phi_{\vec{l'}}\right], \qquad .. \quad (2)$$

$$d\Phi_{\vec{k'}}=dk'_x dk'_y dk'_z, \quad d\Phi_{\vec{l'}}=dl'_x dl'_y dl'_z,$$

where $\vec{F}$ is the total Lorentz force acting on the electron and given by

$$\vec{F}=e\vec{E}+\frac{e}{c}\left[\vec{v}\,\vec{H}\right], .. \qquad .. \qquad .. \qquad .. \quad (3)$$

the group velocity $\vec{v}$ being connected with the wave-vector $\vec{k}$ by

$$m\vec{v}=h\vec{k} \qquad .. \qquad .. \qquad .. \qquad .. \quad (4)$$

and $W\left(\vec{k}\,\vec{k}'\,\vec{l}\,\vec{l}'\right)$ denotes the probability of transition of electrons from state $\vec{k}$ to the state $\vec{k}'$ and ions from $\vec{l}$ to $\vec{l}'$ in unit time and is given by

$$W\left(\vec{k}\,\vec{k}'\,\vec{l}\,\vec{l}'\right) = \left|V_{\vec{k}\,\vec{k}'\,\vec{l}\,\vec{l}'}\right|^2 \frac{\partial}{\partial t} 4 \cdot \frac{\sin^2 \frac{\pi}{h}(\varepsilon'-\varepsilon)t}{(\varepsilon'-\varepsilon)^2} \qquad .. \quad (5)$$

where $V_{\vec{k}\,\vec{k}'\,\vec{l}\,\vec{l}'}$ is the matrix element of the perturbed energy and ε and ε' are energies before and after the process. $f_{\vec{k}}, f_{\vec{l}}$ etc. are the distribution functions of the electrons and ions respectively, the suffixes $\vec{k}, \vec{l}$ standing for their initial and $\vec{k}', \vec{l}'$ for their final states. $\left(1-f_{\vec{k}}\right), \left(1-f_{\vec{l}}\right)$ etc. are introduced to take account of the pre-occupied-ness of the states where the electrons or ions after collisions would go. It follows from the well-known Pauli-principle.

To solve (2), we notice that when the external field is absent, the collision term is zero there being no current in the system and consequently no change in the distribution function. Putting the collision term equal to zero, we should therefore obtain the Fermi distribution function in the absence of a field given by

$$f_0 = \frac{1}{\frac{1}{A} e^{\varepsilon/kT} + 1} \qquad .. \qquad .. \qquad .. \qquad (6)$$

Thus to solve the above equation we put

$$f = f_0 + f_1 \qquad .. \qquad .. \qquad .. \qquad .. \qquad (7)$$

where f_1 represents the change in the distribution function due to the external field and is assumed to be small compared with f_0.

Now to solve for f_1 we put

$$f_1 = \left(\vec{k}\,\vec{\chi}(k)\right) \qquad .. \qquad .. \qquad .. \qquad .. \qquad (8)$$

where $\vec{\chi}(k)$ is a vector depending only on the magnitude of $\vec{k}$.

We now introduce the time of relaxation τ, as defined by

$$\left(\frac{\partial f}{\partial t}\right)_{\text{collision}} = -\frac{f_1}{\tau} \qquad .. \qquad .. \qquad .. \qquad (9)$$

and related to the mean free path l by the relation

$$l = \frac{hk}{m}\tau \qquad .. \qquad .. \qquad .. \qquad .. \qquad (10)$$

Obviously $\tau = \dfrac{1}{\Delta}$, Δ being the number of collisions per second.

Substituting from (6), (7), (8) and (9) in (2), and neglecting the terms arising from f_1, in the presence of those from f_0 we get

$$\left(\vec{k} . \vec{\chi}\right) - \frac{\tau h}{m} \frac{\partial f_0}{\partial \varepsilon} \left[\frac{kT}{A} \left(\vec{k} . \operatorname{grad}_r A\right) + \frac{\varepsilon}{T} \left(\vec{k} . \operatorname{grad}_r T\right) - e\left(\vec{E} . \vec{k}\right) \right.$$
$$\left. + \frac{e\tau}{mc} \left(\vec{k} . \left[\vec{H} . \vec{\chi} .\right]\right) \right) = 0 \quad .. \quad .. \quad (11)$$

so that

$$\vec{\chi} - \frac{\tau h}{m} \frac{\partial f_0}{\partial \varepsilon} \left[\frac{kT}{A} \operatorname{grad}_r A + \frac{\varepsilon}{T} \operatorname{grad}_r T - e\vec{E} \right] + \frac{e\tau}{mc} \left[\vec{H} . \vec{\chi}\right] = 0, \quad (12)$$

since $\vec{k}$ is not zero and $\vec{\chi}$ is independent of the direction of $\vec{k}$.

Equation (12) can be put to the form

$$\vec{\chi} + \vec{Y} + \left[\vec{Z} . \vec{\chi}\right] = 0, \quad .. \quad .. \quad .. \quad (13)$$

where $\vec{Y} = \dfrac{\tau h}{m} . \dfrac{\partial f_0}{\partial \varepsilon}\left[e\vec{E} - \dfrac{kT}{A} \operatorname{grad}_r A - \dfrac{\varepsilon}{T} \operatorname{grad}_r T \right]$ .. .. (14)

$$\text{and} \quad \vec{Z} = \frac{e\tau}{mc} \vec{H} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (15)$$

To solve equation (13) we multiply it scalarly by $\vec{Z}$ and get

$$\left(\vec{Z} . \vec{\chi}\right) + \left(\vec{Z} . \vec{Y}\right) = 0 \quad .. \quad .. \quad .. \quad (16)$$

Again multiplying (13) vectorially by $\vec{Z}$,

$$\left[\vec{Z} . \vec{\chi}\right] + \left[\vec{Z} . \vec{Y}\right] + \vec{Z}\left(\vec{Z} . \vec{\chi}\right) - \vec{\chi}\left(\vec{Z} . \vec{Z}\right) = 0$$

or, applying (13) and (16),

$$-\vec{\chi} - \vec{Y} + \left[\vec{Z} . \vec{Y}\right] - \vec{Z}\left(\vec{Z} . \vec{Y}\right) - \vec{\chi}\left(\vec{Z} . \vec{Z}\right) = 0$$

$$\text{or} \quad \vec{\chi} = - \frac{\vec{Y} + \left[\vec{Y} . \vec{Z}\right] + \vec{Z}\left(\vec{Z} . \vec{Y}\right)}{1 + \left(\vec{Z} . \vec{Z}\right)} \quad .. \quad .. \quad .. \quad (17)$$

§2.   Deduction of a general expression for the current density:—

The current, i.e. the total charge passing through unit area in unit time, is evidently given by

$$\vec{I} = 2e \int\int\int \vec{v} f_1 dk_x dk_y dk_z$$

$$= \frac{2eh}{m} \int\int\int \vec{k}\left(\vec{k} . \vec{\chi}\right) dk_x dk_y dk_z \quad .. \quad .. \quad .. \quad (18)$$

The factor 2 is taken because of the fact that two electrons may occupy the same state with opposite spins.

We shall now assume that the gas is homogeneous and the temperature is constant so that $\mathrm{grad}_r \vec{A}$ and $\mathrm{grad}_r T$ are equal to zero, and from (14)

$$\left. \begin{array}{c} \vec{Y} = \dfrac{\tau e h}{m} \cdot \dfrac{\partial f_0}{\partial \varepsilon} \vec{E} \\[2mm] \text{also } \vec{Z} = \dfrac{e\tau}{mc} \vec{H} \end{array} \right\} \qquad \cdots \quad \cdots \quad \cdots \quad (19)$$

Substituting from (17) and (19) in (18) we obtain the expression for the current which can be easily put to the form [7]

$$\vec{I} = K^t \vec{E} + L^t \left[\vec{E} . \vec{\omega}_L \right] + M^t \vec{\omega}_L \left(\vec{\omega}_L . \vec{E} \right), \qquad (20)$$

where K^t, L^t, M^t are of tensor characters, given by

$$K^t = -\frac{2e^2 h^2}{m^2} \iiint \frac{\vec{k} . \vec{k} \; \tau \; \dfrac{\partial f_0}{\partial \varepsilon}}{1 + \tau^2 \omega_L^2} \, dk_x \, dk_y \, dk_z$$

$$L^t = -\frac{2e^2 h^2}{m^2} \iiint \frac{\vec{k} . \vec{k} \, \tau^2 \, \dfrac{\partial f_0}{\partial \varepsilon}}{1 + \tau^2 \omega_L^2} \, dk_x \, dk_y \, dk_z$$

$$M^t = -\frac{2e^2 h^2}{m^2} \iiint \frac{\vec{k} . \vec{k} \, \tau^3 \, \dfrac{\partial f_0}{\partial \varepsilon}}{1 + \tau^2 \, \omega_L^2} \, dk_x \, dk_y \, dk_z$$

and $\omega_L = $ double of the Larmor frequency $= \dfrac{eH}{mc}$;

$\vec{k} . \vec{k}$ represents the dyodic product of the vectors $\vec{k}$ and $\vec{k}$. Of the nine components of $\vec{k} . \vec{k}$, obviously the diagonal terms remain while the cross terms vanish when integrated over the values of k_x, k_y and k_z.

Further, since the integrals arising from the diagonal terms are equal to one another the expression for the current can be finally reduced to

$$KE + L \left[\vec{E} . \vec{\omega}_L \right] + M \vec{\omega}_L \left(\vec{\omega}_L . \vec{E} \right) \qquad \cdots \quad \cdots \quad (21)$$

where

$$K = -\frac{2e^2 h^2}{3m^2} \iiint \frac{\dfrac{\partial f_0}{\partial \varepsilon} \, k^2}{1 + \tau^2 \omega_L^2} \, dk_x \, dk_y \, dk_z \quad \cdots \quad \cdots \quad (22)$$

$$L = -\frac{2e^2 h^2}{3m^2} \iiint \frac{\tau^2 \, \dfrac{\partial f_0}{\partial \varepsilon} \, k^2}{1 + \tau^2 \omega_L^2} \, dk_x \, dk_y \, dk_z \quad \cdots \quad \cdots \quad (23)$$

$$M = -\frac{2e^2h^2}{3m^2} \iiint \frac{r^3 \frac{\partial f_0}{\partial \varepsilon} k^2}{1+\tau^2\omega_L^2} \, dk_x \, dk_y \, dk_z \quad \cdots \qquad \cdots \quad (24)$$

§3. **Time of relaxation for elastic collisions:**—Since the ions are much heavier than the electrons we shall consider only the case of elastic collisions where no energy is interchanged between an electron and an ion during their encounter. The collision term in (2) reduces therefore to

$$\left(\frac{\partial f}{\partial t}\right)_{\text{collision}} = n^+ \iiint W\left(\vec{k}\,\vec{k'}\right)\left[f_{\vec{k'}} - f_{\vec{k}}\right] dk_x' \, dk_y' \, dk_z' \quad \cdots \quad (25)$$

$$= n^+ \iiint W\left(\vec{k}\,\vec{k'}\right)\left[\left(\vec{k'}\cdot\vec{\chi}(k')\right) - \left(\vec{k}\cdot\vec{\chi}(k)\right)\right] dk_x' \, dk_y' \, dk_z' \quad \cdots \quad (26)$$

where n^+ is the number of ions per c.c.

Although the collision is assumed to be elastic we must integrate over a large number of final states, for all of which k' has nearly the same value as k. In this manner we can arrive at a result of physical significance.

To evaluate the integral we introduce the transformations

$$k_x = k \cos v_x, \ k_x' = k' \cos v_x', \text{ etc.}$$

and also $dk_x' \, dk_y' \, dk_z' = k'^2 \sin \theta \, dk' \, d\theta \, d\alpha,$

where v_x, v_x' are the angles which $\vec{k}$ and $\vec{k'}$ respectively make with the x-axis, θ is the angle between $\vec{k}$ and $\vec{k'}$ and α the angle between the plane of $\vec{k}$, $\vec{k'}$ and the plane of $\vec{k}$ and x-axis.

Integrating, now, over α we obtain

$$\left(\frac{\partial f}{\partial t}\right)_{\text{collision}} = 2\pi n^+ \int_0^\pi \int_0^\infty W\left(\vec{k}\,\vec{k'}\right)\left(\vec{k}\cdot\vec{\chi}(k)\right) k^2 (\cos\theta - 1) \sin\theta \, dk' d\theta.$$

Substituting for $W\left(\vec{k}\,\vec{k'}\right)$ from (5) and changing the variable from k' to ε' we get

$$\left(\frac{\partial f}{\partial t}\right)_{\text{collision}} = \frac{8\pi m}{h^2} kn^+ f_1 \frac{\partial}{\partial t} \int_0^\pi \int_0^\infty (\cos\theta - 1) \sin\theta \left| V_{\vec{k}\,\vec{k'}} \right|^2 \frac{\sin^2 \frac{\pi}{h}(\varepsilon'-\varepsilon)t}{(\varepsilon'-\varepsilon)^2} \, d\varepsilon' \, d\theta$$

$$\cdots \quad (27)$$

Since the function

$$\frac{\sin^2 \frac{\pi}{h}(\varepsilon'-\varepsilon)t}{(\varepsilon'-\varepsilon)^2}$$

has a sharp maximum at $\varepsilon = \varepsilon'$ and is practically zero in other regions we may easily integrate the above by replacing $V_{\vec{k}\,\vec{k'}}$ by its constant value at

$|k'| = |k|$ and changing the limits of ε' to $-\infty$ and ∞. This is however possible provided $t \gg \dfrac{h}{\varepsilon}$. Introducing further a new variable as defined by

$$\xi = \pi \cdot \frac{\varepsilon' - \varepsilon}{L} t,$$

we get

$$\frac{\partial}{\partial t} \int_0^\infty \frac{\sin^2 \frac{\pi}{h} (\varepsilon' - \varepsilon) t}{(\varepsilon' - \varepsilon)^2} \, d\varepsilon' = \frac{\pi^2}{h},$$

so that

$$\left(\frac{\partial f}{\partial t}\right)_{\text{collision}} = - \frac{16 \pi^3 m k}{h^3} n^+ f_1 \int_0^\pi |V_{\vec{k}\,\vec{k}'}|^2 \sin^2 \frac{\theta}{2} \sin\theta \, d\theta \quad .. \quad (28)$$

By comparing with (9) we therefore obtain,

$$\frac{1}{\tau} = \Delta = \frac{16 \pi^3 m k n^+}{h^3} \int_0^\pi |V_{\vec{k}\,\vec{k}'}|^2 \sin^2 \frac{\theta}{2} \sin\theta \, d\theta \quad .. \quad (29)$$

§ 4. We find from equation (21) that the current is composed of three parts, parallel to the electric field, parallel to the magnetic field and perpendicular to the plane of the electric and magnetic fields. The first component represents a radial motion if we assume that the electric field is directed radially and if the magnetic field be that as due to an elementary magnet we find that the electrons will have a general tendency to flock towards the magnetic equator. The stream lines thus resemble, in general features, the long coronal streamers during sunspot minimum. The third component is directed perpendicular to the meridian planes and shows that the stream lines should spiral round the sun.

We shall now study the conductivity in two particular cases of general interest, namely, those of the longitudinal and transverse magnetic fields.

In the first case we have $\left[\vec{E} \cdot \vec{\omega}_L\right] = 0$ and from (21) the current is given by

$$\vec{I} = \vec{E} \left(K + M \omega_L^2\right),$$

So that the conductivity in the direction of the field is equal to

$$\frac{I}{\vec{E}} = K + M \omega_L^2$$

$$- \frac{2 e^2 h^2}{3 m^2} \iiint \tau \frac{\partial f_0}{\partial \varepsilon} k^2 dk_x \, dk_y \, dk_z \quad .. \quad .. \quad (30)$$

On the other hand when the magnetic field is totally absent we have from (21), $\vec{I} = K \vec{E}$, so that the conductivity is equal to K and from (22), putting $\omega_L = 0$, we get the same expression for conductivity as is given by (30). Thus in the presence of a longitudinal magnetic field the conductivity is found to remain unchanged, which is also physically evident; for, the portion of the

Lorentz force due to the magnetic field is $\frac{e}{c}\left[\vec{v}.\vec{H}\right]$ which is obviously zero when the motion $\vec{v}$ and the field $\vec{H}$ are in the same direction.*

In the case of transverse magnetic field where $\vec{E}$ is perpendicular to $\vec{H}$, we obtain from (21)

$$\vec{I} = K\vec{E} + L\left[\vec{E}.\vec{\omega}_L\right] \qquad .. \qquad .. \qquad .. \quad (31)$$

Thus apart from the current in the direction of the electric force $\vec{E}$ there is an additional current perpendicular both to the electric and magnetic fields which is the well-known Hall current.

In general when $\vec{E}$ makes any angle with $\vec{H}$, the current produced is the vector sum of those produced by the components of $\vec{E}$ parallel and perpendicular to $\vec{H}$ when acting separately. Thus it is sufficient to consider the two particular cases separately and as the first case brings about no change in the current and conductivity we shall confine our attention to the case of transverse magnetic field only.

If the magnitudes of the currents in the two directions given by (31) be set equal to $\sigma_1 E$ and $\sigma_2 E$, then σ_1 is the ordinary conductivity of the material, whereas we shall call σ_2 the transverse conductivity.

Thus, from (31), we have

$$\sigma_1 = K, \quad \sigma_2 = L\omega_L, \qquad .. \qquad .. \qquad .. \quad (32)$$

where K and L are given by (22) and (23).

To proceed further we must obtain, at the outset, the expression for τ which is involved in the integrals for K and L. As their proper evaluation by the methods of ordinary kinetic theory met with a serious difficulty of having a divergent integral and was subject to interesting discussions by Chapman and others, we now develope, in the following, a method to obtain τ, which is free from these difficulties.

The Matrix element $V_{\vec{k}\,\vec{k}'}$ which occurs in the expression for τ in the equation (29) is given by

$$V_{\vec{k}\,\vec{k}'} = \int \psi^*_{\vec{k}'} V(r)\psi_{\vec{k}} \, d\tau \qquad (33)$$

where $\psi_{\vec{k}}$, $\psi_{\vec{k}'}$ are the eigenfunctions of the electron before and after the collision and $V(r)$ is the potential field between the electron and the ion, which we assume to be of the form

$$V(r) = \frac{ze^2}{r} e^{-r/b}, \qquad .. \qquad .. \qquad .. \quad (34)$$

---

* It is to be noted that we have taken in our present discussion only the Lorentz force acting on the electron and neglected the effect of quantisation of the motion of electrons in the external magnetic field introduced by Landau. (L. Landau, Zs. f. phys., 64, 629, 1930; S. Titeica, Ann. d. phys., 22, 129, 1935; A. Sommerfeld and W. Bartlett, Phys. Zs., 36, 894, 1935.)

where z is the number of free electrons per atom and b the so-called screening constant which gives the effect of the interaction of the other electrons and ions and is only a small fraction of the average distance between the ions. It can be calculated without much difficulty from Debye-Hückel's theory of electrolytic solution provided the gas is not too dense. Thus if

$$\frac{ze^2}{QkT} < 1, \qquad .. \qquad .. \qquad .. \qquad .. \quad (35)$$

where Q is the mean diameter of the ions, we obtain[8]

$$b^2 = \frac{kT}{4\pi e^2 z(z+1)n^+} \qquad .. \qquad .. \qquad .. \quad (36)$$

$$= 47\cdot9 \, \frac{T}{z(z+1)n^+} \qquad .. \qquad .. \qquad .. \quad (37)$$

With the above value of $V(r)$ we obtain

$$V_{\overrightarrow{k\,k'}} = \frac{4\pi z e^2}{\frac{1}{b^2} + 4\pi^2(|k-k'|)^2}, \qquad .. \qquad .. \qquad .. \quad (38)$$

which for elastic collisions reduces to

$$V_{\overrightarrow{k\,k'}} = \frac{4\pi z e^2}{\frac{1}{b^2} + \left(4\pi k \sin\frac{\theta}{2}\right)^2} \qquad .. \qquad .. \qquad .. \quad (39)$$

From (29) and (39) we have, after integration,

$$\Delta = \frac{1}{\tau} = \frac{2\pi m z^2 e^4 n^+}{h^3 k^3}\left[\log\frac{1+\gamma}{\gamma} - \frac{1}{\gamma+1}\right]$$

where
$$\gamma = \frac{1}{16\pi^2 k^2 b^2}$$

Or, since $\varepsilon = \dfrac{k^2 h^2}{2m}$, we have finally

$$\frac{\pi z^2 e^4 n^+ J}{(2m)^{\frac{1}{2}}\varepsilon^{\frac{3}{2}}} \qquad .. \qquad .. \qquad .. \quad (40)$$

where
$$J = \log(t+1) - \frac{t}{t+1}, \qquad .. \qquad .. \qquad . \quad (41)$$

$$t = \frac{1}{\gamma} = \frac{32\pi^2 m}{h^2}\,\varepsilon b^2 \qquad .. \qquad .. \qquad .. \qquad .. \quad (42)$$

$$= 6,63 \; 10^{27}\varepsilon b^2 \; .. \qquad .. \qquad .. \qquad .. \quad (43)$$

With this value of τ the evaluation of the integrals (22), (23) and (24) is not easily possible without some further simplifications. We therefore carry out the integrations only in the two limiting cases, namely when $\tau\omega_L \ll 1$ and $\tau\omega_L \gg 1$. Changing the variable from k to ε in the integrals (22), (23) and (24) and

remembering that in the atmospheres of the ordinary stars the gas obeys the Maxwellian Distribution Law, so that

$$\frac{\partial f_0}{\partial \varepsilon} = - \frac{A}{kT} e^{-\varepsilon/kT}, \quad A = \frac{nh^3}{2(2\pi mkT)^{\frac{3}{2}}} \qquad .. \qquad .. \quad (44)$$

we obtain, for $\tau\omega_L \ll 1$,

$$K = \frac{e^2}{m} n\bar{\tau} \left(1 - \frac{\overline{\tau^3}}{\bar{\tau}} \omega_L^2 \right) \qquad .. \qquad .. \qquad .. \quad (45)$$

$$L = \frac{e^2}{m} n\,\overline{\tau^2} \left(1 - \frac{\overline{\tau^4}}{\overline{\tau^2}} \omega_L \right) \qquad .. \qquad .. \qquad .. \quad (46)$$

$$M = \frac{e^2}{m} n\,\overline{\tau^3} \left(1 - \frac{\overline{\tau^5}}{\overline{\tau^3}} \right) .. \qquad .. \qquad .. \qquad .. \quad (47)$$

and for $\tau\omega_L \gg 1$, $\quad K = \frac{e^2}{m} n \frac{\overline{\tau^{-1}}}{\omega_L^2} \qquad .. \qquad .. \qquad .. \qquad .. \quad (48)$

$$L = \frac{e^2}{m} n \cdot \frac{1}{\omega_L^2} \qquad .. \qquad .. \qquad .. \qquad .. \quad (49)$$

$$M = \frac{e^2}{m} n \cdot \frac{\bar{\tau}}{\omega_L^2} \qquad .. \qquad .. \qquad .. \qquad .. \quad (50)$$

$$\text{where} \quad \overline{\tau^n} = \frac{\int \tau^n \frac{\partial f_0}{\partial \varepsilon} \varepsilon^{\frac{3}{2}} d\varepsilon}{\int \frac{\partial f_0}{\partial \varepsilon} \varepsilon^{\frac{3}{2}} d\varepsilon} \quad .. \qquad .. \qquad .. \qquad .. \quad (51)$$

Substituting the value of τ from (40) in (51) and by integrating we obtain

$$\bar{\tau} = \frac{2^{\frac{7}{6}} m^{\frac{1}{6}} k^{\frac{2}{3}}}{\pi^{\frac{2}{3}} e^4} \cdot \frac{T^{\frac{3}{2}}}{z^2 n^+ \bar{J}} \quad .. \qquad .. \qquad .. \qquad .. \qquad .. \quad (52)$$

$$\overline{\tau^2} = \frac{315 m k^3}{4\pi^2 e^8} \cdot \frac{T^3}{z^4 n^{+2} \bar{J}^2} \qquad .. \qquad .. \qquad .. \qquad .. \quad (53)$$

$$\overline{\tau^3} = \frac{1920 \times 2^{\frac{1}{2}} m^{\frac{3}{2}} k^{\frac{9}{2}}}{\pi^{\frac{7}{2}} e^{12}} \cdot \frac{T^{\frac{9}{2}}}{z^6 n^{+3} \bar{J}^3} \qquad\qquad\qquad (54)$$

$$\overline{\tau^4} = \frac{675675 m^2 k^6}{16\pi^4 e^{16}} \times \frac{T^6}{z^8 n^{+4} \bar{J}^4} \qquad .. \qquad .. \qquad .. \quad (55)$$

$$\overline{\tau^5} = \frac{960 \times 504 (2m)^{\frac{5}{2}} k^{\frac{15}{2}}}{\pi^{\frac{11}{2}} e^{20}} \times \frac{T^{\frac{15}{2}}}{z^{10} n^{+5} \bar{J}^5} \qquad .. \qquad .. \quad (56)$$

$$\text{and} \quad \overline{\tau^{-1}} = \frac{4\pi^{\frac{3}{2}} e^4}{3(2m)^{\frac{1}{2}} k^{\frac{3}{2}}} \cdot \frac{z^2 n^+ \bar{J}}{T^{\frac{3}{2}}} \qquad .. \qquad .. \qquad .. \quad (57)$$

In the evaluation of the above integrals we have treated J as constant replacing it by its value $\bar{J}$ at $\varepsilon = kT$. This will evidently introduce no significant error, because J is a slowly varying function of energy and the integrand has a sharp rise at $\varepsilon = kT$ due to the exponential factor $e^{-\varepsilon/kT}$ and rapidly falls on both sides, the fall being more rapid for $\varepsilon > kT$.

From equations (45) to (50) we now obtain, for $\tau\omega_L \ll 1$,

$$K = \frac{16n(kT)^{\frac{5}{2}}}{(2m)^{\frac{1}{2}}\pi^{\frac{1}{2}}z^2e^2n^+\bar{J}}\left[1 - \frac{240H^2(kT)^3}{\pi^2mc^2z^4e^6n^{+2}\bar{J}^2}\right] \quad .. \quad .. \quad (58)$$

$$L = \frac{315n(kT)^3}{4\pi^2z^4e^6n^{+2}\bar{J}^2}\left[1 - \frac{2145H^2(kT)^3}{4\pi^2mc^2z^4e^6n^{+2}\bar{J}^2}\right] \quad .. \quad .. \quad (59)$$

$$M = \frac{1920(2m)^{\frac{1}{2}}n(kT)^{\frac{9}{2}}}{\pi^{\frac{1}{2}}z^6e^{10}n^{+3}\bar{J}^3}\cdot\left[1 - \frac{1008H^2(kT)^3}{\pi^2mc^2z^4e^6n^{+2}\bar{J}^2}\right] \quad .. \quad (60)$$

and for $\tau\omega_L \gg 1$,

$$K = \frac{2(2\pi m)^{\frac{1}{2}}z^2e^4c^2nn^+\bar{J}}{3H^2(kT)^{\frac{3}{2}}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (61)$$

$$L = \frac{mc^2n}{H^2} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (62)$$

$$M = \frac{8\times2^{\frac{1}{2}}m^{\frac{1}{2}}nc^2(kT)^{\frac{5}{2}}}{\pi^{\frac{1}{2}}z^2e^4n^+\bar{J}H^2} \quad .. \quad .. \quad .. \quad .. \quad (63)$$

Whence we obtain the conductivities by the relations (32).
Thus for $\tau\omega_L \ll 1$,

$$\sigma_1 = \frac{e^2}{m}n\bar{\tau}\left(1 - \frac{\overline{\tau^3}}{\bar{\tau}}\omega_L^2\right) \quad .. \quad .. \quad .. \quad (64)$$

$$= \frac{16n(kT)^{\frac{5}{2}}}{(2m)^{\frac{1}{2}}\pi^{\frac{1}{2}}z^2e^2n^+\bar{J}}\left[1 - \frac{240H^2(kT)^3}{\pi^2mc^2z^4e^6n^{+2}\bar{J}^2}\right] \quad .. \quad .. \quad (65)$$

$$\sigma_2 = \frac{e^2}{m}n\bar{\tau}^2\left(1 - \frac{\overline{\tau^4}}{\bar{\tau}^2}\omega_L^2\right)\omega_L \quad .. \quad .. \quad .. \quad (66)$$

$$- \frac{315nH(kT)^3}{4\pi^2mcz^4e^6n^{+2}\bar{J}^2}\left[1 - \frac{2145H^2(kT)^3}{4\pi^2mc^2z^4e^6n^{+2}\bar{J}^2}\right] \quad .. \quad (67)$$

and for $\tau\omega_L \gg 1$,

$$\sigma_1 = \frac{e^2}{m}n\cdot\frac{\overline{\tau^{-1}}}{\omega_L^2} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (68)$$

$$- \frac{2(2\pi m)^{\frac{1}{2}}z^2e^4c^2nn^+\bar{J}}{3H^2(kT)^{\frac{3}{2}}} \quad .. \quad .. \quad .. \quad .. \quad (69)$$

$$\sigma_2 = \frac{e^-}{m}n\cdot\frac{1}{\omega_L} = \frac{enc}{H} \quad .. \quad .. \quad .. \quad .. \quad (70)$$

The integrals K, L, M may also be easily evaluated in the special case when $\tau\omega_L$ is near about unity. With the approximation $\tau\omega_L = 1$ we then obtain, from (22), (23) and (24)

$$K = \frac{e^2}{2m}\, n\,\bar{\tau} = \frac{8n(kT)^{\frac{3}{2}}}{(2m)^{\frac{1}{2}}\pi^{\frac{3}{2}}z^2e^2n^+\bar{J}} \qquad .. \qquad .. \qquad .. \qquad .. \quad (71)$$

$$L = \frac{e^2}{2m}\, n\,\bar{\tau^2} = \frac{315n(kT)^3}{8\pi^2z^4e^6n^+{}^2\bar{J}^2} \qquad .. \qquad .. \qquad .. \qquad .. \quad (72)$$

$$\text{and } M = \frac{e^2}{2m}\, n\,\bar{\tau^3} = \frac{960(2m)^{\frac{1}{2}}n(kT)^{\frac{9}{2}}}{\pi^{\frac{1}{2}}z^6e^{10}n^+{}^8\bar{J}^3} \qquad .. \qquad .. \qquad .. \quad (73)$$

Thus, in this case,

$$\sigma_1 = \frac{e^2}{2m}\, n\,\bar{\tau} = \frac{8n(kT)^{\frac{3}{2}}}{(2m)^{\frac{1}{2}}\pi^{\frac{3}{2}}z^2e^2n^+\bar{J}} \qquad .. \qquad .. \qquad .. \quad (74)$$

$$\text{and } \sigma_2 = \frac{e^2}{2m}\, n\,\bar{\tau^2}\,.\,\omega_L = \frac{315nH(kT)^3}{8\pi^2mcz^4e^5n^+{}^2\bar{J}^2} \qquad .. \qquad .. \qquad .. \quad (75)$$

To obtain, therefore, the numerical values of σ_1 and σ_2 at different layers of the solar atmosphere, having different densities and temperatures we must, at the outset, evaluate $\tau\omega_L$ to see which of the approximations may be applicable in any particular layer.

Now $\qquad\qquad\qquad \bar{\tau}\omega_L \ll 1$ amounts to $H \ll \dfrac{mc}{e\bar{\tau}} \qquad .. \qquad .. \qquad .. \quad (76)$

Or, defining a limiting magnetic field H_0 by

$$H_0 = \frac{mc}{e\bar{\tau}} \qquad .. \qquad .. \qquad .. \qquad .. \quad (77)$$

we obtain the condition $H \ll H_0$.

Since, then

$$\bar{\tau} \ll \frac{2\pi mc}{eH}, \text{ i.e. } \ll \frac{2\pi}{\omega_L},$$

the physical significance of the above condition is that the average time between two collisions is small compared with the period of circling round the direction of the magnetic field. The electron should therefore perform only a part of its orbit between successive collisions and the conductivity is necessarily decreased to some degree. On the other hand, if $H \gg H_0$ or $\bar{\tau}\omega_L \gg 1$, i.e. if the collision period $\bar{\tau}$ be large compared with the Larmor period $\dfrac{2\pi}{\omega_L}$ in the magnetic field the electron will make complete orbits before the collision takes place.

The average value of τ is obtained from (52) as

$$\bar{\tau} = 1,89\,\frac{T^{\frac{3}{2}}}{z^2 n^{+}\bar{J}} \quad .. \quad .. \quad .. \quad .. \quad (78)$$

Thus $\bar{\tau}$ and consequently H_0 depend on the density and temperature of the gas.

We shall tabulate here values of H_0 for different values of density and temperature of the solar atmosphere and also the corresponding values of σ_1 and σ_2 by examining in each case the relation between H and H_0. We assume that the gas is singly ionized in all the cases, i.e. $z = 1$.

n^{+}in c.c.	T°C	H(Gauss)	H_0(Gauss)	σ_1E.S.U.	σ_2E.S.U.
5×10^{13}	5,500	150	56·6	$6\cdot1 \times 10^{12}$	$4\cdot8 \times 10^{12}$
$2\cdot5 \times 10^{12}$	5,500	30	3·4	$4\cdot6 \times 10^{11}$	$1\cdot2 \times 10^{12}$
5×10^{13}	5,500	56·6	56·6	$6\cdot3 \times 10^{12}$	$1\cdot2 \times 10^{13}$
$1\cdot7 \times 10^{14}$	9,800	2,000	81	$1\cdot7 \times 10^{11}$	$1\cdot2 \times 10^{12}$
$4\cdot5 \times 10^{14}$	13,000	2,000	138	$7\cdot6 \times 10^{11}$	$3\cdot3 \times 10^{12}$
$1\cdot5 \times 10^{18}$	50,000	2,000	37,360	$5\cdot5 \times 10^{14}$	$5\cdot6 \times 10^{13}$

The first set of values for n^{+} and T corresponds roughly to conditions at the base of the reversing layer, whereas the second set gives the value at a height of 300 km. It is to be noticed that the values of conductivities as obtained by Cowling and Ferraro agree, at least in order of magnitude, with those deduced here. The magnitude of H_0 in our case is, however, always much less than the corresponding value of Cowling and this favours the spiralling of the electrons to begin even near the base of the reversing layer.

§ 5. We shall now study the conductivity along the electric field when the Hall current is stopped in an artificial way as is done in the laboratory. We take the x and z-axis along the electric and magnetic fields respectively so that the Hall current is in the y-direction. If we prevent the Hall current by applying a suitable additional electric field in that direction then the resulting current and conductivity can be obtained from (31) thus:—

We have $\qquad I_x = KE_x + LE_y\omega_L$

and $\qquad I_y = KE_y - LE_x\omega_L.$

Since, in the present case $I_y = 0$, we have

$$I_x = \left[K + \frac{L^2\omega_L^2}{K}\right]E_x$$

$$= \frac{\sigma_1{}^2 + \sigma_2{}^2}{\sigma_1} E_x, \quad \text{using (32)}$$

Thus, if σ denotes the resulting conductivity in the direction of x we have

$$\sigma = \frac{\sigma_1{}^2 + \sigma_2{}^2}{\sigma_1} \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (79)$$

Now expressing this in terms of σ_0, the conductivity in the absence of the magnetic field, which is obviously as found from (65) equal to

$$\sigma_0 = \frac{16n(kT)^{\frac{1}{2}}}{(2m)^{\frac{1}{2}}\pi^{\frac{1}{2}}z^2e^2n+\bar{J}} , \qquad \ldots \quad \ldots \quad \ldots \quad (80)$$

we obtain finally the change of conductivity in magnetic field by substituting the values of σ_1 and σ_2 from (65), (67), (69) and (70). Thus for $\tau\omega_L \ll 1$, we have

$$\sigma_0 \quad \frac{2H^2(kT)^3}{\pi^2mc^2z^4e^6n+2\bar{J}^2}\left[120 - \pi\left(\frac{315}{64}\right)^2\right]$$

$$= 1 - 2\cdot4\times10^{15}H^2\frac{T^3}{z^4n+2\bar{J}^2} \qquad . \qquad \ldots \quad \ldots \quad \ldots \quad (81)$$

And for $\tau\omega_L \gg 1$,

$$\frac{\sigma}{\sigma_0} = \frac{3\pi}{32} + \frac{\pi^2z^4e^6n+2\bar{J}^2mc^2}{12(kT)^3}\cdot\frac{1}{H^2}$$

or

$$\left(\frac{\sigma}{\sigma_0}\right)_{H\to\infty} = 0\cdot29 \qquad \ldots \quad \ldots \quad \ldots \quad (82)$$

We therefore find that the conductivity first decreases with the increase of the impressed magnetic field and evidently quadratically with it and then in very strong field it tends to a limiting value, independent of the magnetic field, leading to saturation. The results are thus in accord with the observations. The difficulty in the treatment of Cowling in explaining the conductivity in the magnetic field when the Hall current is made zero is thus removed.

It is a great pleasure for me to acknowledge my heartiest thanks to Dr. R. C. Majumdar for many interesting and valuable discussions and to Dr. D. M. Bose, the Director of the Institute, and Profs. M. N. Saha and S. N. Bose for their interest and encouragement during the course of the work.

References

[1] S. Chapman—M.N. 89, 59, 1928.
[2] R. Gunn—Phys. Rev. 33, 614, 1929.
[3] V. C. A. Ferraro—M.N. 95, 280, 1935.
[4] S. Chapman—M.N. 89, 59, 1928, also addendum to this paper, p. 79.
[5] V. C. A. Ferraro—M.N. 93, 416, 1933.
[6] T. G. Cowling—M.N. 93, 90, 1932.
[7] D. Blockinzev and L. Nordheim—Zs. f. phys. 84, 168, 1933; M. Bronstein—Phys. zs. d. Sowjet-Union 2, 28, 1932; For a general treatment see R. C. Majumdar—Zs. f. phys. 107, 599, 1937.
[8] R. H. Fowler—Statistical Mechanics, 2nd edition (1936), p. 272; A. S. Eddington—Internal constitution of stars, (1926) Chap. X.

III.   ON THE OSTEOLOGY OF THE MĀLÉR

By S. S. SARKAR

In Vol. X of the Transactions of the Bose Research Institute a few long bones from the Mālé cemetery of *Danowar* (Borio, Rajmahal) were described by the present author (1934-35).   A second lot of long bones was also recovered in February, 1937, from the denser part of the above cemetery which will be described in the present paper.   In March, 1938, a large number of human bones were also recovered from the *Guma Pahar* * cemetery at Durgapur (Rajmahal) and forms till now the largest collection of authentic skeletal remains of the Mālér.   At the latter place remains of more than one single individual were found, of which the larger number belongs to a female.   A skull, a complete pelvic girdle and the two pairs of long bones of the lower extremity can be identified as belonging to the female.   This latter skeleton appear to be a comparatively recent burial since the dried up tissue fibres were still adhering to the bones.

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* The *Guma Pahar* cemetery is situated at a distance of about quarter of a mile from the village on a much higher level than the village.   The author had been to the spot with a Mālé boy (Fig. 1) and a village watchman.   When the skeletal remains were seen the Mālé boy was asked to bring from his village an old basket in which the bone could be carried. The boy returned with a small basket and when he saw the author picking up the bones he at first refused to give the basket.   The villagers could see all what is being done in the cemetery and they all grew curious when the basket was brought.   Further, a woman from the village cried out to the boy something, at which the latter replied.   Immediately the women began to weep loudly and the author was compelled to suspend any further search for the bones, lest anything untoward may happen.   The collected bones were somehow piled upon the basket and a part tied in the cloth of the watchman and the latter was asked to carry it to the Dak Bungalow through the jungle path which does not touch the village.   The author came down to the village and saw the women weeping in a body while the men standing in a row opposite to them.   The author had left the officers of the forest department in the village who had already begun the task of consoling them.   The author explained to the villagers that the bones are not intended for any magical purposes and they will be reburied as they were not properly buried there.   The greatest difficulty was to console a young married girl who had recently lost her mother and was also buried in the same cemetery.

Here too, as in *Danowar*, persons dying of small-pox (Sarkar, 1938) are not buried. Further, remains from a few graves appear to have been taken out by wild animals and it appeared that the dead is not so securely buried here.   This can be very well seen from a burial in the photograph where the dead is buried and covered by an inverted string cot. In other places, large stone boulders are placed after the body is covered with earth.   The rocky nature of the soil of the cemetery of *Guma Pahar* stands in the way of making deep ditches for burials.

FIG. 1.   Mālé Cemetery at *Guma Pahar*, Durgapur, Rajmahal.

For facilities of comparison the measurements of the long bones published before have been incorporated here.

The skeletal remains.

The total human remains from *Danowar* are as follows:—
  (*a*) Humerus—Right and left; upper and lower ends missing.
  (*b*) Ulna—Right, head and lower end missing.
  (*c*) Femur—Right and left; right complete; articular head and neck of the left missing.
  (*d*) Tibia—Right and left; left complete; upper and lower ends of the right missing.
  (*e*) Fibula—Right and left; upper and lower ends of both missing.

The human remains from the *Guma Pahar* cemetery are as follows:—
  (*a*) Skull—Female, complete, parts of the soft bones from the basi-occipital and the palate missing.
  (*b*) Skull—Male, facial portion completely missing, parts of right and left parietals broken.   The skull was found in fragments but reconstruction has been possible due to the presence of the majority of the articular surfaces of the frontal and other bones.
  (*c*) Lower Jaw—Complete; of the teeth only the right second molar was found embedded in the mandible.

(*d*) Scapula—Left; complete, excepting a small end of the acromion
    process.

(*e*) Humerus—Left; complete.

(*f*) Radius—Right; complete.

(*g*) Ulna—Right and left; left complete; lower end of the right missing.

(*h*) Pelvic girdle—Complete with two *Os Coxae* and a sacrum.

(*i*) Femur—(i) Complete right and left of an individual; fits well into
            the sockets of the above pelvic girdle.

         (ii) Right of a second individual; epiphysis worn out.

         (iii) Right of a third individual; only lower third present.

(*j*) Tibia—(i) Right and left of an individual; complete.

         (ii) Left of an individual; condyles worn out and lower
            malleolus missing.

(*k*) Fibula—Left; lower part missing.

(*l*) Astragalus—Left; complete.

The above forms the complete list of the bones which will be described
in the present paper.

The pelvic girdle belongs to a female individual and the two pairs of the
femur and the tibia together with the astragalus and the fibula can be associated
with it as they appear from the well-fitted articulating surfaces of the corres-
ponding bones. It is also not unlikely, that the female skull and the almost
complete lower limb belong to the same individual. Similarly, the left ulna
can be very well articulated at the olecranon process of the left humerus and
they appear to belong to the same individual. The *Danowar* remains from the
general texture of the bones and for reasons mentioned below appear to
belong to the same individual whereas at *Guma Pahar* we have skeletal
remains of at least three individuals. The bones have been described according
to their normal anatomical order. All the measurements have been given in
millimetres.

The skull.

The skulls, one male and one female (Pls. IV & V, Figs. 1–8) found at *Guma
Pahar*, belong to adults. The female skull is complete and is in a better state of
preservation than the male. In the female skull, only a part of the right
zygoma and the malar bones are missing and excepting the absence of a few
soft parts of the palate and the basioccipital, the skull is complete for all
osteometric purposes. The male skull was found in fragments and the
facial part is entirely missing. Even after reconstruction of the fragments, a
large part of the two parietals at the coronal suture were missing. The right
occipital bone has also undergone some amount of flattening at the region of
the parietal tuberosity and though it has been found possible to articulate
the bone at the right lambdoidal suture, interspaces are to be seen at the region
of the right asterion. This has to some extent affected the maximum cranial

breadth of the skull. The frontal bone can also be articulated with the left parietal at the left coronal suture in the region of pterion and with the sphenoid at the right sphenoidal suture. It is possible that the cranial length also has been very slightly affected.

For facilities of comparison, it will be worth while to describe the two skulls together.

Norma verticalis
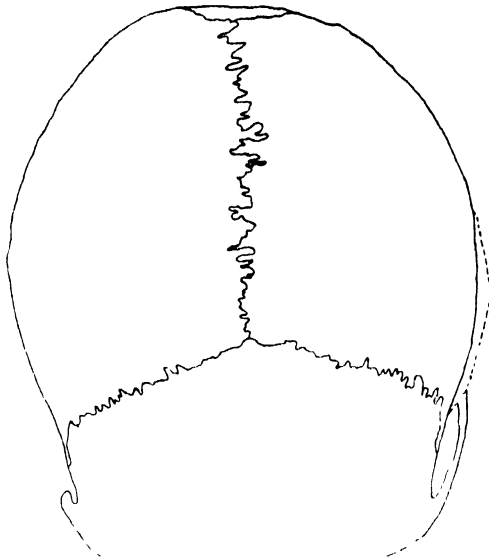
FIG. 2. Vertical view of the Malé ♀ cranium × ⅓.

Viewed from the top (Pl. IV, Fig. 1) both the skulls show an elongated shape which is more marked in the case of the male than the female. The female skull has a narrow and tapering frontal bone which is broad in the male. The difference is, however, very much marked on the occipital side of the two skulls. In the female skull the parietals fall down abruptly and practically nothing of the occipital bone can be seen, while in the male skull the occipital bone bulges out to form a distinct curvature of its own and is also marked by a depressed surface in the region of the lambda.

Norma occipitalis

Viewed from the back (Pl. IV, Fig. 4 & Pl. V, Fig. 8) the female skull shows a characteristic curvature towards the basal surface at the commencement of the superior nuchal line and the area of the muscle attachments is broader than that of the male, though however, the muscular impressions are deeper in the latter.

3B
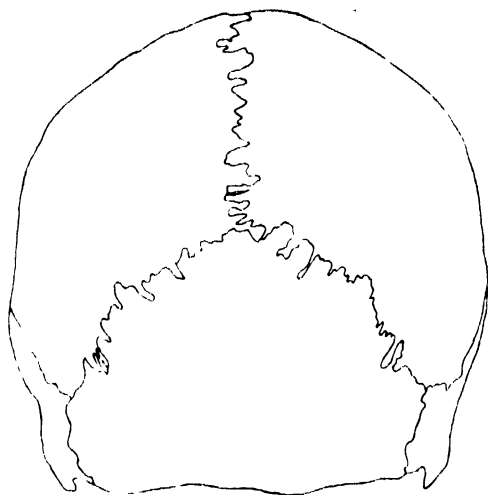
FIG. 3. Occipital view of the Mālé ♀ cranium × ⅓.

The pattern of the lambdoid suture is more complicated in the female than that of the male.

Norma basilaris

At the basal surface, the female skull (Pl. V, Fig. 5) presents a larger occipital area than that found in the male skull (Pl. V, Fig. 6). The male skull, however, shows deeper muscular impressions. The basilar process of the occipital bone is flat and broad in the male while it is rounded and constricted at the middle in the female. To this is due the larger opening of the carotid canal than that of the male. The foramen ovale, on the other hand, is much larger in the male than that of the female. In the male skull there is a foramen at the back of the left occipital condyle, which leads to the jugular foramen. The female skull has a conspicuously large left styloid process. The mastoids are larger in the male, while in the female the points are somewhat curved inwards. The digastric fossa is deeper in the female than that of the male.

The palate is present only in the female skull. All the teeth were erupted and in this skull, only the three left molars, the first left premolar and the first right molar are present. The right molars seem to be more worn out than those of the left.

Norma lateralis

Viewed from the side the male skull (Pl. V, Fig. 7) is characterized by the bulging occiput and the frontal eminences. In the female skull (Pl. IV, Fig. 3) the forehead bends down into a smooth vertical slope whereas the male skull has prominent supraorbital ridges and frontal eminences. Both the skulls are marked by a greater preauricular development, which is however slightly larger in the male than the female. The left styloid process of the female
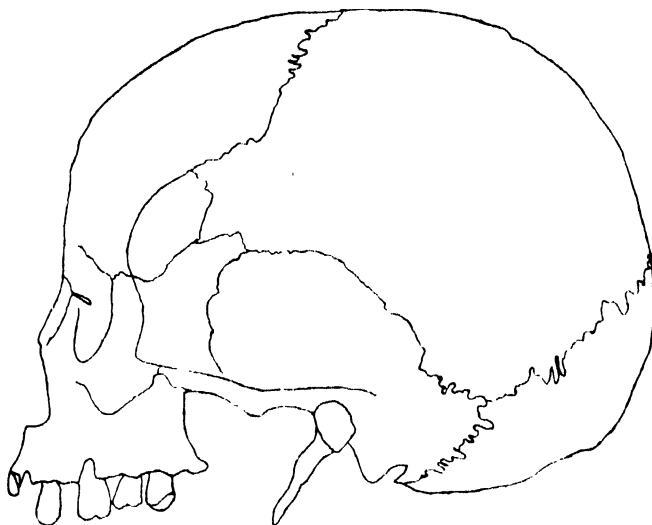
FIG. 4.   Lateral view of the Mālé ♀ cranium × ⅓.

skull is very well preserved and shows an inwardly bent curvature.   The female
skull shows slight alveolar prognathism.
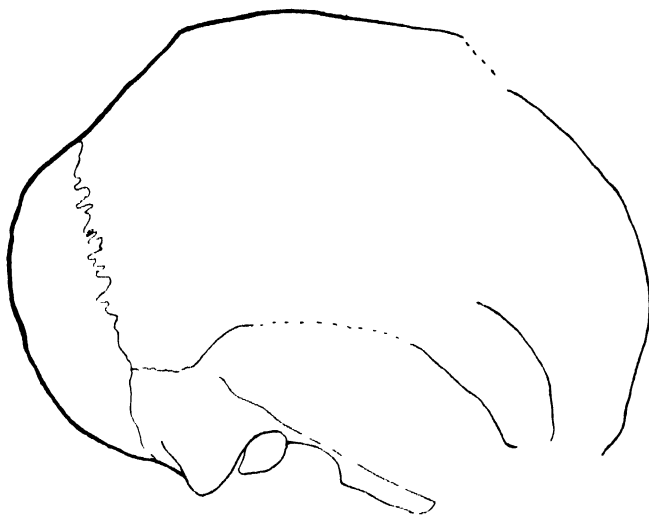
FIG. 5.   Lateral view of the Mālé ♂ cranium × ⅓ (on *n–i* plane).

Norma facialis

The facial portion of the male skull is missing but the peculiarities of the
frontal bone are marked.   The frontal bone presents two eminences; the
glabella and the supraorbital ridges are well marked.   In the female skull
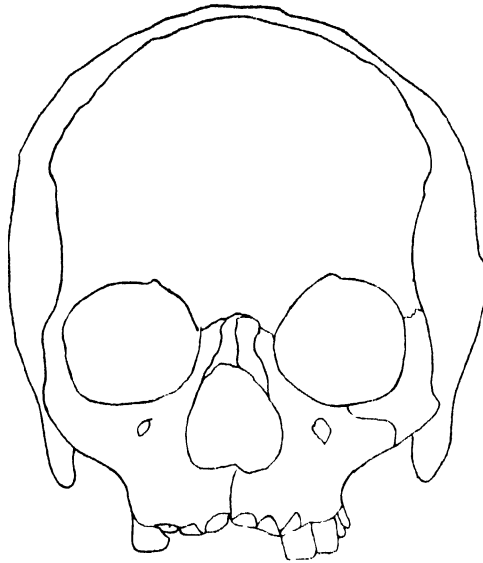
Fig. 6.   Frontal view of the Mālé ♀ cranium × ⅓.

(Pl. IV, Fig. 2) the frontal tuberosities are not well marked. The orbits are circular and the nasal aperture is pyriform. The subnasal spine is prominent. The male skull shows two supraorbital foramina which are in the shape of notches in the female.

Form of the Head.

The male Mālé skull is dolichocranial, the cranial index being 72·46 while the female skull with an index of 79·62 shows a brachycranial tendency. Both the skulls, however, have undergone some amount of postmortem changes, especially the male, and it appears that they have affected the cranial indices to a certain extent. Both the skulls are low-vaulted; the auricular heights measured by Davidson Black's Calottemeter with the skulls placed on Mollison's craniophore are 105 mm. for the female and 107 mm. for the male.

The cranial forms of the two skulls fairly agree with the averages for living subjects measured by the present author (1935-36). The average cephalic index of the Mālér males derived from 188 individuals * was 74·54 while the average of the females derived from the measurements of 7 individuals was found to be 78·59. This gives not only a mesocephalic value but when the 7 individuals are considered separately the actual number of people having a brachycephalic index was 42·86%. The true character of the female head

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* No individuals from Durgapur, however, were measured, as it lies on the margin and it was thought that other strains might have entered into this region.

cannot, however, be definitely judged from such a small sample. The 7 cephalic indices. are as follows:—81·50, 68·54, 75·82, 81·65, 76·24, 74·86 and 80·12. Of the seven 3 are definitely brachycephalic whereas the latter was found to be 1·06% among the males.

Cranial Capacity.

The cranial capacity of the Mālé female skull is 970 c.c. only. I have estimated it firstly by mustard seeds * and the three readings are as follows:—

> 1st Reading   ..   960 c.c.
> 2nd Reading ..   960 c.c.
> 3rd Reading ..   970 c.c.

This gives a mean capacity of 963 c.c. In order to check this result the absolute capacity was determined by water after the whole skull was covered with paraffin wax. With the usual controls necessary in such an experiment the cranial capacity was found to be 970 c.c. only.

TABLE I

Cranial Measurements

Skull	Mālé	Mālé	Mundā (Basu)	Veddā (Sarasin)
Sex	♂	♀	♀	♀
1. Maximum cranial length	167	157	165	179
2. Maximum cranial breadth	121?	125	112	123
3. Nasion inion length	148	148	148	..
4. Basilo-bregmatic height ..	.	118	130	..
5. Least frontal breadth	91·5	91	88	.
6. Greatest frontal breadth ..	111	100	99	.
7. Bimastoid breadth	99	100	94	..
8. Bizygomatic breadth	..	..	116	108
9. Nasion basion line	92	95	98	91
10. Prosthion basion line	.	85	94	..
11. Nasion gnathion line	..	..	..	98
12. Nasion prosthion line	..	52	56	56·5
13. Nasal length ..	..	41	42	41·5

* The graduated glass cylinder, into which the mustard seeds were put, is fitted with a wooden stopper at the mouth through which a wooden piston is allowed into the cylinder to press the seeds. This apparatus belongs to the Anthropological Laboratory of the Indian Museum, Calcutta, and was manufactured by Andrew H. Baird of Edinburgh. The cranial capacities of the skulls measured by Gupte in *Craniological Data from the Indian Museum, Calcutta, 1909*, were determined with the help of this apparatus. In measuring the cranial capacity with mustard seeds I have followed Mollison (*Spezielle Methoden anthropologischer Messung, Handbuch der biologischen Arbeitsmethoden, Abt. VII, Teil 2, Heft 3, 1938*, p. 626) though the seeds were poured into the skull with the help of an ordinary glass funnel used in chemical laboratories instead of the metal one suggested by him.

TABLE I—*contd.*

Cranial Measurements (contd.)

Skull	Mālé	Mālé	Mundā (Basu)	Veddā (Sarasin)
Sex	♂	♀	♀	♀
14. Nasal breadth	..	25	25	23·75
15. Interorbital breadth	.	20	19	19
16. Orbital breadth—				
Right	..	34	35	..
Left	..	..	35	..
17. Orbital height—				
Right	..	31	29	..
Left	..	31	30	..
18. Maxilloalveolar length	..	..	49	..
19. Maxilloalveolar breadth	..	55	62	..
20. Palatal length	..	..	49	..
21. Palatal breadth	..	35	37	..
22. Occipital foramen—				
Length	33	33	31	..
Breadth	28	25	27	..
23. Auricular height	107?	105	..	126·5
24. Sagittal cranial arc	344	330	335	367
25. Transverse cranial arc	.	283	268	.
26. Horizontal circumference	462	455	455	
27. Biauricular breadth	104	103	104	..
28. Outer biorbital breadth	..	96	98	..
29. Inner biorbital breadth	..	90	99	..
30. Greatest occipital breadth	96	94	90	..
31. Frontal arc	..	120	113	..
32. Parietal arc	..	117	120	..
33. Occipital arc	107	93	102	..
34. Frontal chord	..	101	99	..
35. Parietal chord	..	101	107	..
36. Occipital chord	85	77	87	..
37. Length of 1st Molar (Upper)—				
Right { Anteroposterior	..	10	9	..
{ Transverse	..	11	11	..
Left { Anteroposterior	..	10	9	..
{ Transverse	..	10	11	..
38. Length of 2nd Molar (Upper)—				
Right { Anteroposterior	..	..	9	..
{ Transverse	..	..	11	..
Left { Anteroposterior	..	8	9	..
{ Transverse	..	10	11	..
39. Biorbitonasal arc	..	97	100	..
40. Nasion lambda line	154	153	158	..
41. Calvarial height	99	95	100	..
42. Lambda calvarial height	68	67	66	..
43. Bregma position line		87·5	89	..
44. Frontal perpendicular	..	25	24	..
45. Parietal perpendicular	..	25	22	..
46. Occipital perpendicular	26	21	23	..
47. Frontal inclination angle	..	60°	65°	..
48. Occipital inclination angle	86°	89°	87°	..
49. Facial profile angle	.	80°	84°	..
50. Calvarial base angle	.	10°	10°	..
51. Frontal curvature angle	.	125°	130°	..
52. Parietal curvature angle	..	127°	135°	..

TABLE I—*concld.*

Cranial Measurements (concld.)

Skull	Mālé ♂	Mālé ♀	Mundā (Basu) ♀	Veddā (Sarasin) ♀
Sex	♂	♀	♀	♀
53. Occipital curvature angle..	117°	124°	125°	..
54. Occipital flexional angle ..	118°	124°	125°	..
55. Superior facial length angle	..	35°	32°	..
56. Nasion to foot of bregma perpendicular	..	51	41	..
57. Cranial capacity in c.c. ..	..	970 (Estimated)	1100 (Calculated)	1175 (Estimated)
Indices of the cranium.				
1. Length breadth index	72·46	79·62	67·88	68·7
2. Length height index	..	75·16	78·79	70·7
3. Breadth height index	..	94·40	116·07	..
4. Calvarial height index ..	66·89	64·19	67·57	..
5. Bregma position index ..	..	34·46	27·70	..
6. Sagittal cranial curvature index	43·02	44·85	44·17	..
7. Transverse cranial curvature index ..	..	22·64	38·80	..
8. Transverse fronto-parietal index ..	75·61	72·80	78·57	..
Indices showing the relations of the various Sagittal arcs.				
1. Fronto-parietal index ..	..	97·50	106·19	..
2. Fronto-occipital index ..	..	77·50	90·27	..
3. Parieto-occipital index ..	..	79·49	85·00	..
4. Fronto-sagittal arc index..	..	36·36	33·73	..
5. Parieto-sagittal arc index..	..	35·45	35·82	..
6. Occipito-sagittal arc index	31·10	28·18	30·44	..
Indices showing the amount of curvature (bulging) of each of the three contour bones of the cranium.				
1. Frontal curvature index ..	..	84·17	74·44	..
2. Parietal curvature index ..	..	86·32	89·17	..
3. Occipital curvature index ..	79·44	82·80	85·29	..
Indices of the Face.				
1. Total facial index	..	..	..	..
2. Superior facial index ..	..	..	48·27	..
3. Zygomatico frontal index	..	..	75·86	..
4. Orbital index—				
Right ..	..	91·18	82·85	..
Left ..	..	..	..	..
5. Nasal index ..	..	60·98	59·52	57·2
Indices showing relations between cranium and face.				
1. Longitudinal cranio-facial index ..	..	54·14	34·57	..
2. Transverse cranio-facial index ..	..	..	103·57	..
3. Vertical cranio-facial index ..	..	44·07	43·07	..
Some additional indices.				
1. Lambda calvarial height index ..	44·16	43·79	41·51	..
2. Frontal perpendicular index ..	..	24·75	24·24	..
3. Parietal perpendicular index ..	..	24·75	20·56	..
4. Occipital perpendicular index ..	30·59	27·27	26·44	..

TABLE II

Measurements of the face in the anteroposterior plane (Mālé, ♀ skull)

Projection of the lateral orbital margin before mid-auricular plane	..			..	61
Projection of the malo-maxillary point		..	..	..	..	58
Projection of the nose	..	..	..	..	..	75
Projection of the ascending nasal process of maxilla		..	..	..	..	73
Projection of the lateral nasal margin		..	..	..	..	70
Projection of the nasion	..	..	..	..	..	69
Projection of the pre-auricular	..	..	..	..	..	71
Projection of the subnasal point	..	..	..	..	..	75
Projection of the upper alveolar point	..	..	..	..	78

Lower Jaw.

The lower jaw (Pl. VI, Figs. 9 & 10) found at the *Guma Pahar* cemetery belongs to an old individual. It shows the presence of the right second molar only, which is also very much worn out. The process of absorption is marked in the lower jaw, specially on the left side. The socket of the second left molar is almost absorbed. The mental foramina have nearly approached the alveolar border. The lower jaw is characterized by the presence of prominent superior genial tubercles.

The following are the measurements of the lower jaw :—

Bicondylar breadth	.. ..	116
Bigonial breadth	.. ..	92
Leng. of the ramus	.. ..	50
Max. breadth of the ramus	..	37
Min. breadth of the ramus	..	27
Symphyseal height	.. ..	27
Mandibular length	.. ..	57·5
Mandibular angle	.. ..	132°

Indices

Mandibular Index	.. ..	49·57
Ramus Index	.. ..	54·0

The measurements of the right second molar are :—

Anteroposterior length	..	11
Transverse length	.. ..	11

Scapula.

The left scapula (Pl. VI, Figs. 11 & 12) was recovered from the *Guma Pahar* cemetery and belongs to an adult individual. The acromion process of the spine is missing and small portions of cancellous tissues are exposed at the inferior angle and at the terminus of the spinal axis upon the vertebral border

in the supraspinous region.   There is also a deep suprascapular notch in the scapula.   The measurements of the scapula are as follows:—

Measurements

Morphological length (max. br.)	85
Morphological breadth (max. leng.)	118?
Spinal axis	86
Length of the supraspinous line	45?
Length of the infraspinous line	84?
Ant. post. diameter of the glenoid fossa (vert.)	32
Dorsoventral diameter of the glenoid fossa (trans.)	21
Length of the axillary border	116?

Angles

Spinal axis angle	101°
Infraspinous angle	86°
Vertebral border angle	94°
Axillospinal angle	47°

Indices

Scapular index	72·03?
Supraspinous index	38·14?
Infraspinous index	71·19?
Axillary index	98·31
Fossorial index	53·57
Glenoid index	65·63

Humerus.

The complete left humerus (Pl. VII, Fig. 13c) was found from the *Guma Pahar* cemetery while the other two bones (Pl. VII, Figs. 13a & b) with the epiphysis missing were recovered from the *Danowar* cemetery. All the three bones possess slight curvatures at the upper end of the shaft, which is marked in the left humerus of the two obtained from *Danowar*. The bones from the two cemeteries show some notable variations. The *Danowar* bones, as will be evident from the circumference measurements of the shafts, are stronger than that found from *Guma Pahar*. The *Guma Pahar* humerus is characterized by a long and deep bicipital groove and the muscular impressions can be very sharply distinguished from one another. The bicipital grooves in both the *Danowar* bones are flat and the shafts are rounded in appearance whereas it is somewhat triangular in the *Guma Pahar* specimen. The *Danowar* bones

seem to be without doubt belonging to the right and left sides of the same individual.

The measurements of the humerii are given below:—

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TABLE III

Measurements

</div>

Measurements	Guma Pahar	Danowar	
	Left	Right	Left
Maximum length	271		
Physiognomic length	274		
Breadth of the prox. epiphysis	40		
Breadth of the distal epiphysis	54		
Longitudinal diameter of the head ..	40		
Transverse diameter of the head ..	37		
Circumference of the shaft at upper 3rd ..	50	60	59
Least circumference of the shaft ..	48	58	56
Circumference of the head	120		
Indices			
Caliber index	17·20		
Index of the head	92·50		
Angles			
Torsion angle	144·5°		
Cubital angle	79·0°		

Radius and Ulna.

Only one radius (Pl. VII, Fig. 15) of the right side and two ulnæ (Pl. VII, Fig. 16), one right and one left, were found from the *Guma Pahar* cemetery whereas the shaft fragment of a right ulna was recovered from *Danowar*. The lower end of the right ulna from *Guma Pahar* is missing. All the bones are characterized by their curvatures in the shafts; the ulnar curvatures being marked along the upper third of the bones.

The peculiarity of the ulna lies in the development of the interosseous border. In the *Danowar* specimen the ulna does not show the presence of any sharp crest but a triangular border is marked at the upper third of the shaft. In the *Guma Pahar* specimens the crests are prominent no doubt but they begin 67 mm. downwards from the lower lip of the radial notch in the right and 37 mm. downwards in that of the left side. The *Danowar* specimens are stouter and stronger than the *Guma Pahar* bones.

In the radius the interosseous border is also prominent; the crest measures only 60 mm. long and begins 15 mm. below the tuberosity. The curvature of the bone is also a point of interest. The measurements of the radius and the ulnæ are given below:—

Measurements of the Radius

	Right
Max. length	218
Physiological length	206
Least circumference of the distal half ..	30
Sagittal diam. of the shaft	10
Transverse diam. of the shaft	10
Ht. of the perpendicular on the greatest curva-	
ture of the shaft	6

Indices

Caliber index	14·56
Diaphyseal index	41·43
Curvature index	4·06

Angle

Collo-diaphysial angle	164°

Measurements of the Ulnæ

Measurements	Guma Pahar		Danowar
	Right	Left	Right
Max. length	..	231	..
Physiological length	..	207	..
Least circumference of diaphysis.. ..	28	29	38
Max. breadth of the olecranon cap ..	20	19	..
Ht. of the olecranon cap	2	3	..
Thickness of the olecranon cap ..	15	14	.
Indices			
Caliber index	..	14·03	..
Olecranon cap index	..	1·45	..
Curvature index	..	2·38	..
Angle			
Joint Axis Angle	..	81°	..

Pelvic Girdle.

The pelvic girdle (Pl. VIII, Fig. 17) comprising of the two *os coxæ* and the sacrum (without the coccyx), was found from the *Guma Pahar* cemetery in an excellent state of preservation. The pelvis presents distinctive characters of the female sex and the two femora can be associated with it also.

That the pelvis belongs to a female is evident from the following facts:—

The articular surface of the sacrum does not extend more than two sacral vertebrae, the triangular obturator foramen, the presence of the pre-auricular

sulcus, the everted ischial tuberosities, vertical ilia, the small acetabula and the shallow and wide ischiatic notches. The muscular impressions are not prominent. The feminine character of the bone is further borne out from the measurements * given below. The sacrum is short and wide. The upper part is flat while the lower end is bent inwards. The breadth of the sacrum is 98 mm. while the length is 97 mm. The sacral index is 101·03 while the coxal index is 76·51.

Measurements of the Pelvis

Measurements	Māle
A. External dimensions	
Breadth of the pelvis	231
Height of the pelvis	166
Br. between ant. sup. iliac spines	196
Br. between pos. sup. iliac spines	69
Br. between ischial tuberosities (outer)	131
Vert. dia. of the acetabulum	46
Trans. dia. of the acetabulum	44
Vert. dia. of the obturator for.	41
Trans. dia. of the obturator for.	32
Breadth height index	71·86
Obturator index	78·05
Subpubic angle	71°
B. Dimensions of the cavity of the true pelvis	
Trans. diameter of the brim	111
Conjugate diameter of the brim	103
Oblique diameter:—	
Right	111
Left	111
Inferior Sagittal diameter	92
Inter tuberal diameter	95
Depth of the pubic symphisis	30
Depth of the pelvic cavity	81
Pelvic index	92·79
C. Dimensions of the individual bones	
Length of the ilium	108
Breadth of the ilium	127
Br. of the innominate bone	144
Length of the os pubis	76
Length of the ischium	66
Iliac index	117·59
Pubic index	59·84
Ischiatic index	39·16
Innominate index (Turner)	86·75

Femur.

Of all the skeletal remains of the Mālér the femora (Pls. VIII & IX, Figs. 18 & 19) are the largest in number. In 1935 two femora were found from *Danowar* and in the *Guma Pahar* cemetery three more complete femora and the

* In taking the measurements of the pelvis I have largely followed the methods of Turner (Challenger Reports, XVI, 1886).

lower half of another femur were found. The *Danowar* femora were described in an earlier publication. The measurements and the other important features are also noted here for comparative purposes. For the sake of brevity the femora have been numbered as follows:—

A—Right and left, correlated with the pelvic girdle described above, *Guma Pahar*. (Pls. VIII & IX, Figs. 18 & 19A.)

B—Right side, *Guma Pahar*. (Pls. VIII & IX, Figs. 18 & 19B.)

C—Lower half of the right side, *Guma Pahar*. (Pls. VIII & IX, Figs. 18 & 19C.)

D—Right and left from *Danowar*.

Femora A have been correlated with the pelvis because the head of the femora articulates very well in the acetabular cavity. The femur B appears to belong to a female also but the lighter colour of the bone and the sign of attrition present in the two extremities of the femur show that it has no relationship with the pelvis. The femur fragment numbered C appears to belong to a male. The *Danowar* femora have also been identified to be belonging to a female.

Of the female femora the *Danowar* bones appear to be stouter and stronger than the pair from *Guma Pahar* (A). The *Danowar* femora show the presence of a well-developed *crista aspera* whereas it is not so prominent in the *Guma Pahar* remains. The gluteal tuberosities are prominent in the *Guma Pahar* bones while the hypotrochanteric fossa is present in the form of a shallow area in both the two specimens. This hypotrochanteric fossa is very well marked in the B femur. The B femur, though belonging to a more slender individual than the *Danowar* one, agrees very much with the latter. The ridge-like formation in the texture of the *linea aspera* is almost identical; the latter presents the highest pilastric index of 128·57. That the fragment of the femur, numbered C, appears to be of a male is evident from its wide popliteal surface, the well-developed lower part of the *crista aspera* with its prominent medial and lateral supracondylar lines and the deep, wide intercondylar notch and the bicondylar width.

The measurements of the femora are given as follows:—

TABLE IV

Measurements

Measurements	A		B	C	D	
Sex	Rt.	Lt.	Rt.	Rt.	Rt.	Lt.
A. Length						
bsolute length	392	388	396	..	399	..
hysiological length	388	387	392	..	387	..
rochanteric length	366	368	372	..	375	.
iaphysial length ..	330	331	338	..	362	369

TABLE IV—*contd.*
Measurements (contd.)

Measurements	A		B	C	D	
Sex	Rt.	Lt.	Rt.	Rt.	Rt.	Lt.
B. Shaft						
Prox. dorso-ventral diameter	20	21	22	..	23	23
Prox. medio-lateral diameter	25	26·5	23	..	28	28
Medial dorso-vent. diameter	24	24	27	27	27	29
Medial medio-lateral diameter	22	22	21	23	26	26
Circumference of shaft	72	73	75	..	81	83
C. Proximal end						
Oblique proximal length	76	77	..	..	75	..
Leng. of head and neck	59	58	55	..	54	..
Vert. diameter of head	36	37	.	..	37	..
Trans. diameter of head	35	36	..	..	34	..
Circumference of head	114	115	.		112	..
Vert. diameter of neck	25	24	25		25	..
Trans. diameter of neck	23	23	22	.	29	.
Circumference of neck	78	77	75	.	86	..
D. Distal end						
Dorso-ventral diameter of the shaft just above the condyles	23	24	28	26	26	26
Medio-lateral diameter of the shaft just above the condyles	29	31	32	39	31	29
Greatest medio-lateral breadth across the epicondyles	66	64	..	69	48	
Greatest dorso-ventral length of the lateral condyle	54	52	49	54	47	42 5
Greatest dorso-ventral length of the medial condyle	49	48		56	53	.
Bicondylar width	66	65	.	72	56	
E. Angles						
Collodiaphysial angle	131°	130°	135°		125°	..
Condylodiaphysial angle	100°	99°	98°		75°	..
Angle of torsion	32°	25·5°	28°		35°	v
Indices						
A. Caliber						
Length circumference index	18·56	18·86	19·13	.	20 96	
Length diameter index	11·86	11·89	12·24	.	11·11	.
B. Shape						
Platymeric index	80·00	79·25	95·65	..	82·14	82 14
Pilastric index	109·09	109·09	128·57	.	103·85	111·54
Popliteal index	79·31	77·42	87·50	66·67	83·87	89·69
C. Indices of the proximal end						
Head index	97·22	97·30	..	..	91·89	.
Robusticity index	18·30	18·86	..	..	18·35	
Neck length index	15·21	14·99	14·03	.	13·95	

TABLE IV—*concld.*

Measurements (concld.)

Measurements	A		B	C	D	
Sex	Rt.	Lt.	Rt.	Rt.	Rt.	Lt.
D. Indices of the distal end						
Epicondylar breadth index ..	17·01	16·49	..	..	12·40	..
Intercondylar index ..	90·74	92·31	..	103·71	112·77	..
Condylar length index ..	13·92	13·44	12·50	..	12·14	..

All the femora except B are platymeric, the average index being 80·88; the femur B with an index of 96·65 is eurymeric. The subtrochanteric flattening is marked in the A femora and at this region both the femora present a slight curvature at the lateral side which is however not marked in any other femur belonging to this collection.

Tibia.

Altogether three tibiae (Pls. X & XI, Figs. 20 & 21) have been found from the *Guma Pahar* cemetery, of which one pair appears to belong to one individual, while the third belongs to the left side of another individual. A right tibia was also found from *Danowar*, and this along with the left tibia found from the same place in 1935 and described before, completes the pair. As in the case of the femora, the tibiae have also been similarly numbered. The pair of tibiae belonging to the same individual, found from *Guma Pahar* has been numbered A, the singular left one from the same site B and the pair from *Danowar* D.

From the articular surfaces of the condyles of the two pairs of femora and tibiae from *Guma Pahar* one is tempted to associate the two pairs of bones to one individual. The tibiae from the two cemeteries show only minor differences. The *Danowar* bones are stronger than the *Guma Pahar* ones. The B tibia appears to belong to a young adult individual. The tibiae A is characterized by the presence of a well-developed soleal line—the upper end of which forms an oblique ridge-like formation along the medial border and is continued up to the middle of the shaft. In the *Danowar* bones it is moderately present and is continued only up to the level of the nutrient foramen.

The measurements of the tibiae are as follows:—

TABLE V

Measurements

Measurements	A		B	D	
Sex	Rt.	Lt.	Lt.	Rt.	Lt.
Length					
Maximum length (sp-mall.) ..	333	332	..	..	339
Maximum length (cond-mall.)	332	330	..	..	336
Physiological length	316	315	326	..	321
Shaft					
Dorso-ventral diameter (prox.)	36	36	34	40	39
Medio-lateral diameter (prox.)	25	25	22	26	23
Dorso-ventral diameter (med.)	28	28	30	32	32
Medio-lateral diameter (med.)	18·5	18	19	20	19
Dorso-ventral diameter (dist.)	24	26	26	28	28 5
Medio-lateral diameter (dist.)	18	18	17	19	19
Circumference of shaft (med.)	65	67	68	77	76
Least circumference of shaft	..	64	63	70	70
Prox. epiphysial breadth .	62	63	.		65
Sagittal diameter of dist. epiphy.	31	31	..	.	31
Angles					
Retroversion angle ..	10°	10°	..		20°
Inclination angle	5°	4°	..	..	14·5°
Indices					
Platycnemic index ..	66·07	64·29	63·33	62·50	59·38
Caliber index .	..	19·28	.	..	20·65
Femoro-tibial index .	84·95	85·57	.	..	

Fibula.

Two shaft fragments of the fibulæ (Pl. XII, Fig. 22), one from *Danowar* and the other from *Guma Pahar*, were found. Shaft fragment of a fibula was also found from *Danowar* in 1935. The *Danowar* fibula is very much stronger than the *Guma Pahar* specimen. The *Danowar* specimen is exactly similar to that described in my earlier paper and similarly possess a deep groove for the tibialis posterior muscle.

Astragalus.

The left astragalus (Pl. XII, Figs. 23 & 24) was found from the *Guma Pahar* cemetery. The bone shows only one peculiarity at its plantar aspect. The *sulcus tali* is very much narrowed down by the approaches of the posterior calcanear articular surface and the middle calcanear articular surface at its lateral border.

The measurements of the astragalus are as follows:—

Maximum length	.. ..	49
Maximum breadth	.. ..	36
Maximum height	.. ..	26
Length of the trochlear	.. ..	29
Breadth of the trochlear	.. ..	22
Length of the head	.. ..	23
Breadth of the head	.. ..	18
Length of the post ant. facet for calcaneous	.. .. ..	31
Breadth of the post ant. facet for calcaneous	.. .. ..	19
Leng.-br. index	.. ..	73·47

Affinities.

Due to the paucity in general of authentic skeletal remains belonging to the Indian aborigines and the fact that our present materials consist of only two skulls of each sex no far-reaching and definite conclusions regarding the racial affinities of this tribe can be made. Basu (1932-33, 1933-34) has already described 7 Mundā and 4 Orāon crania in earlier publications of the Transactions of this Institute and his results are available for comparison with our data. A Veddā-Australoid strain has long been stressed in the aboriginal population of India and the Veddā measurements published by the Sarasin Brothers and other workers have, therefore, been utilized here for comparison. In Table VI the measurements of the male Mālé skull are compared with the average figures of the Mundā, Orāon and Veddā.

TABLE VI

	Orāon (4 crania) ♂	Mālé (1 cranium) ♂	Mundā (4 crania) ♂	Veddā (18 crania) ♂
Cranial length	182·75	167	179	180·2
Cranial breadth	130·5	121?	130	126·9
Nasion-inion length	174	148	167	
Least fr. breadth ..	91·75	91·5	90·75	89·9
Greatest fr. breadth	113	111	109·25	107·5
Bimastoid breadth	99·5	99	99	.
Nasion-basion line	98·75	92	97	.
Sagittal cranial arc	370·75	344	365·5	362
Hor. circumference	507·5	462	501·5	..
Biauricular br.	116·5	104	112·5	..
Greatest occ. br.	104·5	96	104·5	..
Caanial index ..	71·48	72·46	72·64	70·5

Mālé and Mundā ♂.

As already stated, only a limited number of measurements could be taken on the male Mālé skull, owing to the complete absence of the facial region

4B

and the fragmentary nature of the skull cap. The male Mālé skull has a maximum cranial length of 167 mm. and a maximum cranial breadth of 121 mm. against the average cranial length and breadth respectively of 179 mm. and 130 mm. of the Mundā skulls measured by Basu (1932-33). As is to be expected the Mālé cranium shows a nasion-inion length of only 148 mm. against 167 mm. of the Mundā skull.

The difference in the size of the skull is still more clearly brought out when the sagittal cranial arc and the horizontal circumference are compared but the shape in both the Mālé and the Mundā crania is closely similar as seen from the respective figures for the cranial index which are 72·46 and 72·64. The difference in size between the two series need not, however, be stressed as we have only one Mālé skull to judge from.

Mālé and Orāon ♂.

As compared with the Mālé, the average cranial length and breadth of the Orāon skulls (Basu, 1933-34) are 182·75 mm. and 130·5 mm. respectively showing differences of 15·75 mm. and 9·5 mm. respectively from the Mālé skull. As is to be expected the nasion-inion length of 174 mm. in the Orāon crania is 26 mm. greater than the corresponding figure of 148 mm. in the Mālé skull. The larger size of the Orāon skull is further shown by the differences in the sagittal arc and the horizontal circumference which are 370·75 mm. and 507·5 mm. respectively in the Orāon against 344 mm. and 462 mm. in the Mālé. In cranial shape however, the two series are very similar; the average cranial index being 72·46 in the Mālé against 71·48 in the Orāon.

Mālé and Veddā ♂.

Compared with the Veddā averages of 180·2 mm. and 126·9 mm. for cranial length and breadth respectively, as given by the Sarasin Brothers (1892-93), the Mālé skull shows also lower values for the cranial measurements. The difference of 13·2 mm. in the cranial length between the Veddā and the Mālé closely follows the differences of 15·75 mm. and 12 mm. found respectively in the case of the Orāons and the Mundās. In head breadth the Mālé skull stands nearer to the Veddās than the Chota Nagpur tribes. The difference between the Mālé and the Veddā head breadths is 5·9 mm., compared to 9·5 mm. and 9 mm. respectively between it and the Orāon and the Mundā respectively. The difference between the Mālé and the Veddā in sagittal cranial arc is 18 mm. compared with 26·75 mm. and 21 mm. respectively between it and the Orāons and the Mundās. As with the Mundās and the Orāons, the cranial index of the Mālé skull closely agrees with that of the Veddā. No significant racial difference can therefore be said to have been revealed between the Mālé and the other cranial series in spite of the undoubted smallness in the size of the former.

The paucity of identified skeletal materials for Indian aborigines is even more apparent in the case of female crania. I am not aware of published

measurements of any Orãon female crania and we have available for purposes
of comparison the only well-preserved Mundã crania No. 607 of the Indian
Museum collection, the measurements of which have already been published by
Basu.   There are also some figures on Veddã female crania published by the
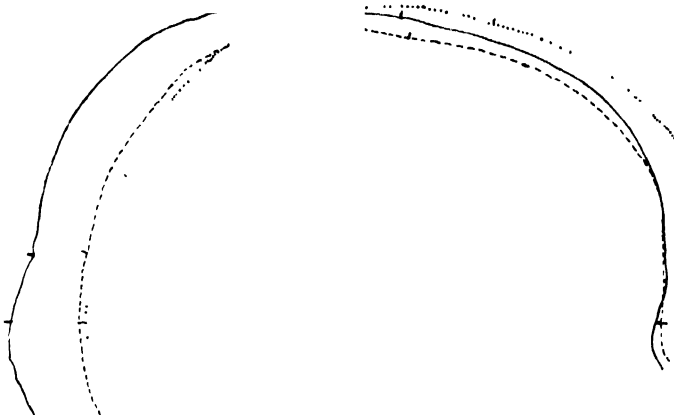Sarasin Brothers.   In Fig. 7 are transposed the median sagittal craniograms

FIG. 7.   Median sagittal craniograms of ♀
——Veddã (Sarasins),   - - - - Mãlé, ... Mundã crania × ½.

of the female Mãlé skull, the female Veddã skull taken from the craniogram
published by the Sarasins (Pl. LXIX, Fig. 134) and the female Mundã skull
mentioned above.   It will be seen from this that the Mãlé cranium is the smallest
of the three.   While the Mundã skull is remarkable for its forward growth, the
posterior parts are best developed in the Veddã skull.   The Mãlé skull agrees
very closely in the shape of the frontal part with that of the Veddã but differs
widely in the occipital region.   In the latter part it, however, agrees fairly
closely with the Mundã skull, but not in its forward projection of the whole
frontal and facial parts.   There is also some agreement between the Mãlé
and the Veddã skulls in the conformation of the nasal parts.

In the absence of any comprehensive female cranial data I have
undertaken here a comparison of the three individual crania mentioned
in fig. 7.

Mãlé and Veddã (♀).

The female Mãlé cranium with a cranial index of 79·62 (cranial leng.
157 mm.; cranial br. 125 mm.) shows a tendency towards brachycrany while
the Veddã skull with an index of 68·7 (cranial leng. 179 mm.; cranial br. 123 mm.)

is distinctly dolichocranial. The difference between the two crania is more marked in the length (22 mm.) than that in the breadth (2 mm.). In auricular height also the difference is marked, the respective figures being 126·5 mm. and 105 mm. in the two skulls. The Sarasins took only a limited number of measurements which I have reproduced in Table 1. Of these the sagittal cranial arc of the Veddā skull is found to be 367 mm. as against 330 mm. of the Mālé skull.

In the built of the nose, however, there is a close coincidence. The nasal length is 41·4 mm. in the Veddā as against 41 mm. in the Mālé, while nasal breadths of the Veddā and the Mālé are 23·75 mm. and 25 mm. respectively. The nasal indices calculated from the above figures are 57·2 for the Veddā as against 60·98 in the Mālé. The two crania thus agree with one another in the form of the nose.

Mālé and Mundā (♀).

The single female Mundā skull available for comparison is dolichocranial having a cranial index of 67·88 (cranial leng. 165 mm.; cranial br. 112 mm.) * as against 79·62 of the Mālé (cranial leng. 157 mm.; cranial br. 125 mm.). The relation between the lengths and breadths of the two crania is just the reverse of what we found between the Mālé and the Veddā. In the present case the difference in cranial length is only 8 mm. compared with that of 22 mm. found between the Mālé and the Veddā. In cranial breadth the difference between the Mālé and the Mundā is 13 mm. as against 2 mm. between the Mālé and the Veddā. There is a remarkable coincidence in nasion-inion length which is 148 mm. in both the crania. In the measurements, the two skulls are not very much widely differentiated except in the transverse cranial arc which is 283 mm. in the Mālé as against 268 mm. in the Mundā. The horizontal circumference is 455 mm. in both the crania while the sagittal arc shows only a difference of 5 mm. the value being 330 mm. in the Mālé as against 355 mm. of the Mundā. The frontal arc of the Mālé skull is 120 mm. compared with 113 mm. of the Mundā, while the parietal arc is 117 mm. in the former as against 120 mm. of the latter. The occipital arc measurements show somewhat greater variation than the other arc measurements. It is 93 mm. in the Mālé as against 102 mm. in the Mundā. Thus we find that though the two crania agree in many respects there are striking differences in the form and shape of the skull.

As in the case of the Veddā skull the form of the nose in both the two crania is very much similar. Both the skulls have the nasal breadth of 25 mm. while the nasal length is 41 mm. in the Mālé as against 42 mm. in the Mundā. The nasal indices are 60·98 and 59·52 in the Mālé and the Mundā respectively.

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* The cranial breadth of the other broken female Mundā skull No. 611 belonging to the Indian Museum collection is, according to Basu, 123 mm., thus agreeing closely with the cranial breadth of the Mālé skull (125 mm.).

Coming to the angle measurements of the craniograms of the two crania the largest differences are met with in the inclination angle of the frontal bone, this being 60° in the Mālé as against 65° in the Mundā. This is well supported by the curvature angle of the frontal bone which is 130° in the Mālé as compared with 125° in the Mundā. The parietal curvature angle is also greater in the Mundā (135°) than that of the Mālé (127°). The facial profile angle is 84° in the Mundā compared with 80° of the Mālé. The calvarial base angle is 10° in both the crania.

We thus find that the two crania agree with one another in a large number of characters though they differ in their general shape and form. These differences are mostly due to the marked forward growth of the Mundā skull. This becomes clear from the meatal position indices of the two crania, calculated after the method of Sewell and Guha (1931). The Mundā skull has a meatal position index of 53·70, according to Basu, as against 57·14 of the Mālé skull.

Arising out of the above analyses there are some points which require special mention, e.g. the low cranial capacity of the Mālé skull, which is 970 c.c. only. Thomson (1890) has recorded the cranial capacity of 960 c.c. in a female Veddā skull whereas the Sarasins found the lowest capacity of 990 c.c. in the skull of a fifteen year old Veddā girl whose cranial dimensions are larger than those of the Mālé skull. The cranial length of the above-mentioned skull, according to the Sarasins, is 171 mm. and the breadth 112 mm., giving an index of 65·5. Thomson has given the cranial index of the Veddā skull, mentioned above, as 69·9, with a cranial length of 166 mm. and breadth of 116 mm. Among the aborigines of India Martin (1928) has referred to the occurrence of low cranial capacities, such as 950 c.c. and 970 c.c. among the Andamanese and the Kurumbars but I have not been able to verify this statement. Flower (1884) has given the lowest cranial capacity for the Andamanese males to be 1,120 c.c. and that for females as 1,040 c.c. In his earlier paper Flower (1879) found the minimum cranial capacity in an Andamanese female skull to be 1,025 c.c.

Anthropologists have not paid so much attention on other parts of the skeleton as they have to the skull and it may be partly due to this fact that racial values have not been so definitely demonstrated on them. Eugen Fischer, however, as early as 1906, in his famous paper on the variations of radius and ulna, urged the necessity of monographic treatment of every individual bone of the human skeleton. In the preceding pages it has been my object to show the variations in the respective bones and below a small note on the racial variations of the scapula and pelvis has been appended.

The scapular index and the infraspinous index as a racial character was first pointed out by Broca (1878) and then by Flower and Garson (1879). The scapular index of the single Mālé scapula very nearly approaches the Negroes. This being 72·03 in the latter, compared with 68·16 (Broca), 71·7 (Flower and Garson) and 69·7 (Turner) for the Negroes. Turner (1886) has found the highest scapular index of 70·2 among the Andamanese from a study

of 27 scapulae. On the other hand, the low infraspinous index of the Mālé scapula is remarkable. In this case both the scapular indices are found to be almost equal which may be peculiar to this individual, otherwise it has been known that the infraspinous index is always much higher than the scapular index. Martin (1928) has drawn attention to the fact that the scapular measurements are subject to changes due to the working of the muscles in this region though he remarks the differences between the European and Negro averages of the above two indices as '*ein immerhin beachtenswerter Unterschied*'. He lays somewhat more stress on the infraspinous index than the scapular index. With regard to the latter, Martin believes that there can be hardly any racial difference as the bone is subject to two '*heterogene momente*', one due to the broadening of the scapular plate and the other due to the bending of the spine.

The pelvic index of the Mālé female pelvis is 92·79 and the sacral index is 101·03. According to Turner's classification this pelvis falls into the mesatipellic group, which according to him includes the Negroes, Tasmanians, New Caldonians, and Melanesians (?), while the sacrum in the platyhieric group. The platyhieric group includes among others, the Negroes, Europeans, Hindus, etc.

The pelvis of the female Veddā, whose cranium has been compared before together with the median sagittal craniogram given in Fig. 7 and the cranial measurements given in Table I, has, according to the Sarasins, a pelvic index of 95·8 and a breadth-height index of 86 compared with 92·79 and 71·86 respectively of the Mālé. The Sarasins have given the mean pelvic index of 88·2 for the Veddā female, derived from the measurements of 3 individuals while the mean breadth-height index for the same is 78·3.

It will be difficult to say whether the peculiarities of the Mālér skeletal remains are racial or only individual characters as it is very difficult to say from such a meagre data, and the present data will be useful to future workers, who may be fortunate to come across more material, for comparative purposes.

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BIBLIOGRAPHY

1. Basu, P. C.—The Racial Affinities of the Mundas, *Trans. Bose Res. Inst.*, VIII, 1932-33.
2. Basu, P. C.—The Racial Affinities of the Oraons, *Trans. Bose Res. Inst.*, IX, 1933-34.
3. Broca, P.—Sur lés indices de largeur de l'omoplate chez l'hommeles singes et dans la serie des mammiferes, *Bull. Soc. D' Anthropologie*, Paris, I, 3, 1878.

4. Fischer, E.—Die Variationen an Radius und Ulna des Menschen, *Zts. f. Morph. u. Anth.*, IX, 1906.
5. Flower, W. H. & Garson, J. G.—The Scapular Index as a Race character in Man, *Jr. Anat. & Physiology*, XIV, 1879.
6. Flower, W. H.—Osteology and Affinities of the Natives of the Andaman Islands, *Jr. Anth. Inst.*, IX, 1879 & XIV, 1884.
7. Martin, R.—Lehrbuch der Anthropologie, II, Jena, 1928.
8. Sarasin, P. & F.—Ergebnisse Naturwissenschaftlicher Forschungen auf Ceylon, Die Weddas von Ceylon und die sie umgebenden Volkerschaften, Bd. III, Wiesbaden, 1892-93.
9. Sarkar, S. S.—Human Remains from a Mālé Cemetery, *Trans. Bose Res. Inst.*, X, 1934-35.
10. Sarkar, S. S.—The Racial Affinities of the Inhabitants of the Rajmahal Hills, *Trans. Bose Res. Inst.*, XI, 1935-36.
11. Sarkar, S. S.—The Mālér of the Rajmahal Hills, Calcutta, 1938.
12. Sewell, R. B. S. & Guha, B. S.—Report on the Human Remains excavated at Mohenjo-daro and the Indus valley civilization in Mohenjo-daro, edited by Sir John Marshall, Chap. XXX, 607, London, 1931.
13. Thomson, A.—On the Osteology of the Veddahs of Ceylon., *Jr. Anth. Inst.*, XIX, 1890.
14. Turner, W.—Report on the Human Crania and Other Bones of the Skeletons collected during the Voyage of H.M.S. *Challenger*, Vol. XVI, Pt. XLVII, 1886.

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4,

Fig. 5.
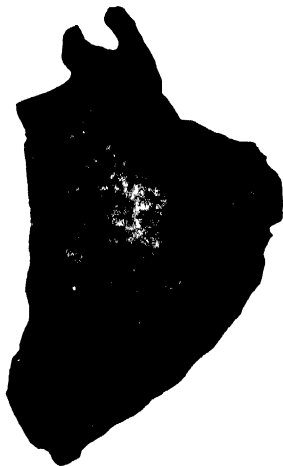
Fig. 6.

Fig. 7.

Fig 8.

FIG. 9.

FIG. 10.

FIG. 11.

FIG. 12.

EXPLANATIONS OF PLATE VII

Fig. 13a.   Lt. Humerus (about $\frac{1}{4}$ nat. size) from *Danowar*.
Fig. 13b.   Rt. Humerus (about $\frac{1}{5}$ nat. size) from *Danowar*.
Fig. 13c.   Lt. Humerus (about $\frac{1}{4}$ nat. size) from *Guma Pahar*.
Fig. 14a-c.   Ventral views of the above.
Fig. 15.   Rt Radius (about $\frac{1}{2}$ nat. size) from *Guma Pahar*.
Fig. 16a.   Rt. Ulna (about $\frac{1}{4}$ nat. size) from *Danowar*.
Fig 16b.   Rt. Ulna (about $\frac{1}{2}$ nat. size) from *Guma Pahar*.
Fig. 16c.   Lt. Ulna (about $\frac{3}{4}$ nat. size) from *Guma Pahar*.

a   *b*   *c*

FIG. 13.

a   *b*

FIG. 14.

FIG. 15.

c   *b*   *a*

FIG. 16.

EXPLANATIONS OF PLATE VIII

Fig. 17.   Pelvic Girdle (about ⅔ nat. size) from *Guma Pahar*.
Fig. 18A. Rt. & Lt. Femora (about ¼ nat. size) from *Guma Pahar*.
Fig. 18B. Rt. Femur (about ⅓ nat. size) from *Guma Pahar*.
Fig. 18C. Rt. Femur (about ¼ nat. size) from *Guma Pahar*.

FIG. 17.
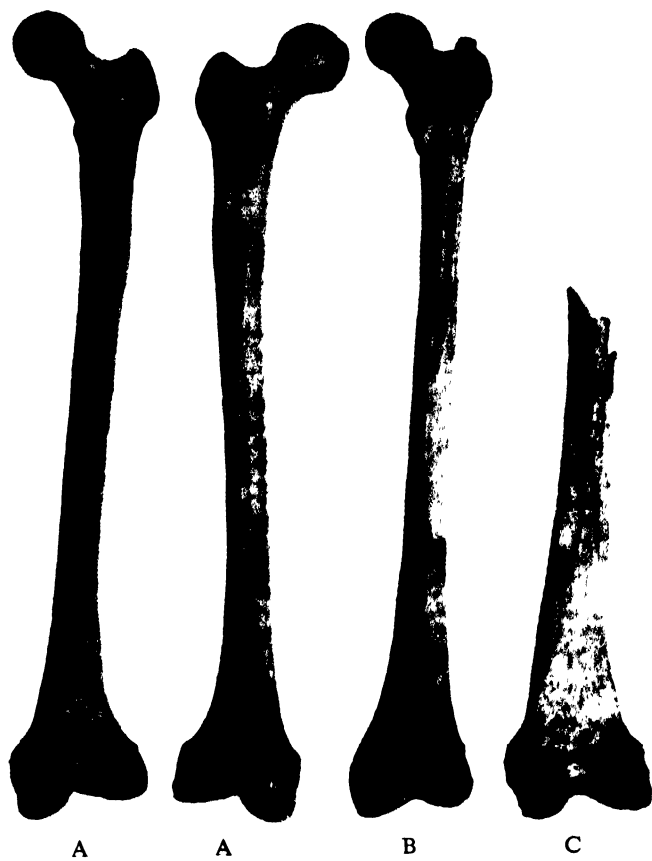
A   A   B   C

FIG. 18.

A   A   B   C

FIG. 19.

C   B   A   A

FIG. 20.

C   B   A   A
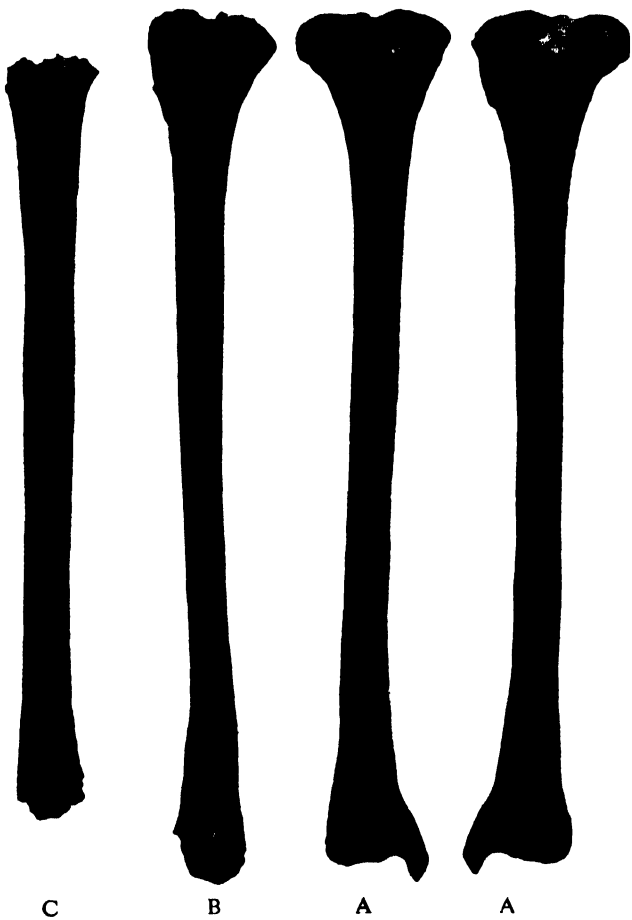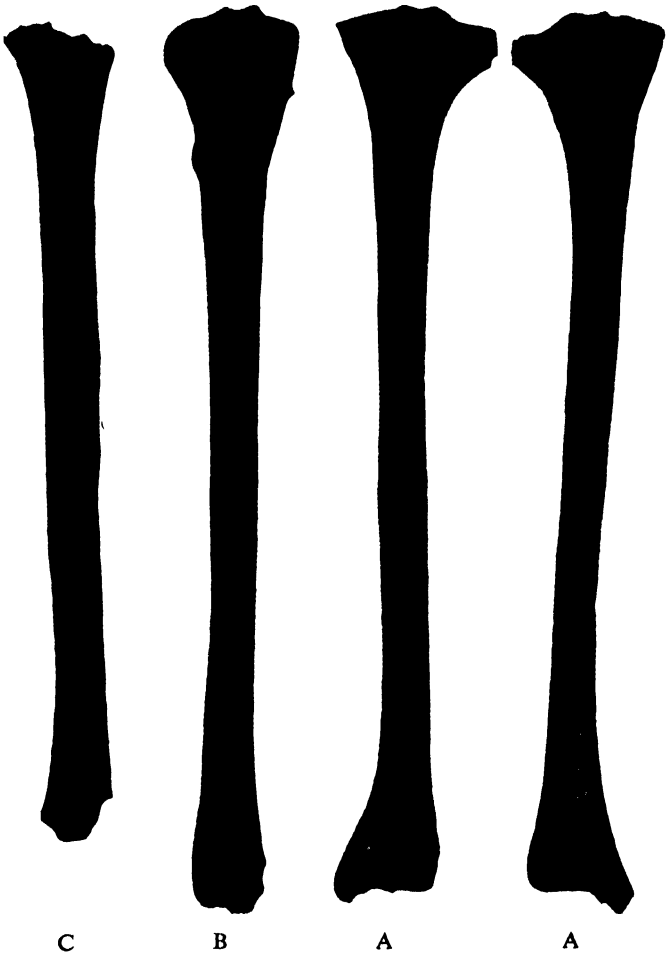
FIG. 21.

EXPLANATIONS OF PLATE XII

Fig. 22a. Shaft fragment of fibula (about ⅓ nat. size) from *Guma Pahai*.
Fig. 22b. Shaft fragment of fibula (about ⅖ nat. size) from *Danowar*.
Fig. 23.   Lt. Astragalus (about nat. size) dorsal view, from *Guma Pahar*.
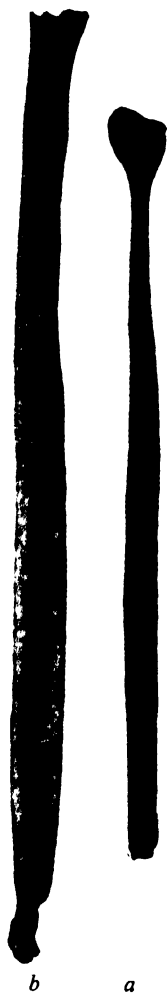Fig. 24.   Lt. Astragalus (about nat. size) ventral view, from *Guma Pahar*
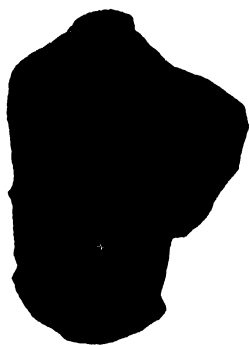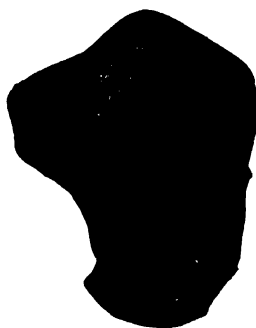
b   a
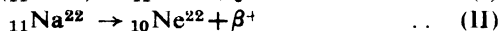
FIG. 22.

FIG. 23.

FIG. 24.

IV. RADIOACTIVE $_{11}Na^{22}$ FROM FLUORINE BOMBARDED BY POLONIUM α-PARTICLES

By N. K. SAHA, M.Sc., D.Sc., P.R.S.

§1. INTRODUCTION: STATEMENT OF THE PROBLEM

The element Fluorine consists of a single isotope $_9F^{19}$ in its natural state of occurrence; yet it is well known that when bombarded by α-particles from Polonium (5·3 MeV energy), this element shows a 'branched' reaction consisting at least of two independent nuclear processes, one with proton (p-) and the other with neutron (n-) emission. A detailed study of these reactions has already been made by the present author [1] by investigating the nature of the p- and the n-emissions and their excitation-functions. The object of the present paper is to report some further studies in connection with the n-emission process. This process can be expressed as

$$_9F^{19} + _2He^4 = (_{11}Na^{23}) \rightarrow _{11}Na^{22} + _0n^1 \qquad .. \qquad .. \qquad (1)$$
$$_{11}Na^{22} \rightarrow _{10}Ne^{22} + \beta^+ \qquad .. \qquad (11)$$

in which (I) indicates that a neutron is emitted by the capture of an α-particle by the F^{19}-nucleus and the resulting nucleus is Na^{22}. But as Na^{22} is energetically unstable, it emits a β^+-particle and is converted into stable $_{10}Ne^{22}$. This is denoted by the reaction (II). Although the neutron-emission process (I) can be directly studied by means of a boron-lined ionization chamber in conjunction with 'Schleifenozillograph', the formation of the positron-active Na^{22} in this process also affords an alternative evidence for the neutron-emission. In fact, it was from the observation of the radioactivity of Na^{22} and its chemical identification that the n-emission process from F^{19} was first discovered by Frisch [2].

The study of radioactivity of Na^{22} is important also from some other points of view. It was early recognized by Frisch that the activity of the product element obtained by bombarding F^{19} by α-particles possesses a particularly long half-life (longer than 6 months) and the half value thickness of the emitted radiation in Al is ∼0·030 gm./cm.², indicating a moderately high energy. Later Laslett [3] obtained Na^{22} from a different nuclear reaction: $_{12}Mg^{24}(d, \alpha)_{11}Na^{22}$, and measured the half value period of its activity at about 3·0 years and a maximum energy of the positrons ∼0·58 MeV. Instances of such very long life β-activity with energies of this order of the emitted β-particles is extremely rare. Among the natural radioactive elements there are some nuclei like $_{82}ThB^{212}$ (10·6 hr.) and $_{82}RaB^{214}$ (26·8 min.) which emit β-particles of similar hardness as those from Na^{22} mentioned above, but the half-lives of their

5

activities are much shorter than in the case of Na^{22}. On the other hand, nuclei like $_{88}MsTh_1^{228}$, $_{82}RaD^{210}$ (0·035 MeV) and $_{89}Ac^{227}$ possess very long life β-activities (several years in each case) but the energies of the β-particles emitted by them are certainly much lower than those from Na^{22}. There is no artificially produced radioactive nucleus * definitely known whose half-life exceeds one year and where the β-rays possess an energy-limit of this order. The light nucleus $_{11}Na^{22}$ occupies, therefore, a unique position in this respect and seems to fall in the group of some rarer isotopes (see §4) where the β-emission involves a forbidden transition. It remains, however, to be verified whether the nuclear spin-change involved in the β-transition for Na^{22} is really large, as any forbidden nuclear transition would demand.

In view of the importance attached to the half-life of the β-activity and the maximum energy of the emitted particles, it has been thought desirable to produce Na^{22} from F^{19} bombarded by Po-α-particles, as was originally done by Frisch, and to ascertain the half-life and the maximum energy by as careful measurements as possible, and thus to check the results of Frisch and of Laslett in an independent way. The experimental difficulty of measuring such long life activity with sufficient accuracy lies in maintaining the constancy of the conditions under which the measurements are made for such a long period. The precautions adopted for eliminating this difficulty together with other experimental details of the methods of measurement will be described in §2.

The importance of determining the maximum energy of the positrons from Na^{22} is also clear. First of all this determination will enable us to fix the position of Na^{22} on the Sargent curve, which would approximately give us an indication of the nuclear spin-change involved in the transition resulting in the positron-emission. Secondly, the very long life of Na^{22} raises the important question of capture of an orbital K-electron by this nucleus as an alternative process to the positron-emission. The theoretical probability of this process can be calculated only when the upper limit of the positron-spectrum and the corresponding spin-change occurring in the nuclear transition are definitely known. A determination of the upper limit of the positron-energy as accurately as possible is therefore thought desirable.

This could of course be done by obtaining the entire energy distribution curve of the positrons by means of cloud-chamber photographs under a magnetic field or by using a β-ray spectrograph in conjunction with point-counters. But the estimation of the upper-limit from the distribution curves involves the assumptions of the Fermi- or the Konopinski-Uhlenbeck theory of β-disintegration regarding which the position to-day is not very certain [4] †. An absorption method of determining the range of the positrons in Al was

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* Except perhaps $_{27}Co^{58}$ ($\sim$2 years).

† See, for example, the interpretation of the β-ray spectrum of $_{33}As^{76}$, N. K. Saha, Naturwissenschaften, 27, 786, 1939; Trans. Bose Res. Inst., 13, 159, 1939.

therefore adopted (§3) in which the extrapolation of the maximum energy seems to be on surer ground (§3).

Finally, from the observed positron-intensity of Na^{22} at the beginning of its decay, the absolute positron yield of Na^{22} per million α-particles is estimated taking into account the various geometrical and other factors which limit the observed positron-intensity (§4). The importance of this yield with regard to the question of the capture of an orbital K-electron by Na^{22} is discussed. The maximum energy of the positrons determined experimentally also enables one to find a lower limit to the mass of $_{11}$Na^{22}. This is attempted in §4 and its numerical value is discussed with reference to the energy-conditions necessary for the positron-emission and the K-capture processes (§4).

§2. EXPERIMENTAL

The experimental procedures adopted for the production and measurement of the radioactivity of Na^{22} and determination of the maximum energy of the emitted positrons can be briefly described under three heads:—

(a) *Preparation of the Polonium-Source.*—A pure β-ray free Polonium (RaF) source was prepared by 'purely chemical deposition with chemical purification' starting from 40 spent Radon-tubes (each on the average 1 cm. long and 0·1 mm. inner diameter). The tubes were from 9 to 15 months old and their Radon-content varied from 20 to 50 millicuries per centimeter length. The chemical method employed was an adoptation of Hafstad's [5] modification of the original Curie-method [6], which in brief consists of the following steps: (1) Digestion of the crushed Radon-bulbs in aqua regia, (2) removal of the copper and alkali-salts by adding NH_4OH, (3) precipitation as sulphides the elements RaD, RaE and RaF and Hg (deposited in the Radon-bulbs from the Hg-diffusion pump of the collecting plant) by passing H_2S in their soluble chlorides, (4) removal of Hg as soluble $Hg(SK)_2$, and (5) dissolving the residue in aqua regia. From this the final solution containing RaD, RaE and RaF is deposited on a Ag-disc ($\sim$8 mm. in diameter and 0·1 mm. thick) by taking advantage of the favourable electrochemical potential of RaF with respect to Ag, the Ag-disc being kept in continual rotation in the solution by means of a clock work.

Throughout the complicated analytical separation the track of the active substance was followed by means of a point-counter (to be described later) which proved to be exceptionally suitable for this purpose. The β-activity of the Po-deposit on the Ag-disc after proper washings was also tested by means of the point-counter, and was found to be very small, although the mother solution left behind showed a strong β-activity.

The α-ray strength of the Po-source was tentatively tested by means of a Geiger-Müller tube counter (with a $\sim$2·4 cm. air-equivalent mica window) set up in the proportional region. Indication of a good α-intensity was

obtained. The final measurement of the Po-strength was done by measuring the ionization current produced by the Po-α-particles placed on the lower plate of a parallel plate ionization-chamber the upper plate of which was ~4·1 cm. vertically above it. A steady potential of ~1,500 Volts was applied to the upper plate from a Medicus Stabiliser. The voltage-drop produced by the ionization-current across a resistance of 10^5 ohms. was measured by means of a potentiometer arrangement using a Hartmann-Braun galvanometer (voltage sensitivity ~ a microvolt). In this way an ionization current of ~6·3 · 10^{-8} amperes was measured which corresponds to a Polonium-strength of 0·4 millicuries [7].

Efficiency of deposit.—The maximum theoretical Po-content of our Radon-seeds can be calculated on detailed consideration of the rate of decay and equilibrium conditions of the whole chain of products from Radon to RaF and it comes out to be 0·5 millicuries in the equilibrium stage (which is reached in nearly 2 years). Allowing for the possibilities that saturation of the ionization current might not have been quite reached in our measurement and our Radon-seeds are on the average only one year old, we can perhaps conclude that an efficiency of nearly 80% has been attained in the Po-deposit. The large efficiency may be due to a not too fast rate of revolution (38 revolutions per minute) of the Ag-disc maintained for a long time (four and a half hours) adopted here, while a very fast rotation (~60 revolutions per minute) continued for a few minutes is the usual orthodox procedure.

(*b*) *Production of Radioactive Na²² and Measurement of its half-life.*—A small crystal of Fluorspar (CaF_2) about $10 \times 10 \times 5$ mm.[3] in dimension was covered with the thinnest possible pure gold-foil available and irradiated in vacuum for 50 days by means of the Po-α-particles by placing the Po-source directly on the covered crystal surface. After this period the Po-source was withdrawn, the gold-foil carefully removed and the activity of the crystal surface examined by means of a point-counter (see below). A fairly good activity was observed which was evidently due to Na^{22} produced by the capture of an α-particle by the F^{19}-nucleus and emission of a neutron.

The Point-Counter.—A brass-ebonite point-counter 58 mm. long, 34 mm. in inner diameter and 1 mm. thick was used. It had an opening with ~10 mm. diameter which was covered with a mica sheet of ~4·7 cm. air-equivalent thickness. The voltage (~1,500 V) necessary for the counter was supplied from a Medicus-stabiliser and was steady within 0·1 per cent. The voltage-count characteristic was carefully examined. By adjusting the distance of the 'point' from the mica-window the counter was so calibrated that (1) all the β-particles incident normally on the window at its different points were counted with equal efficiency, and (2) the particles incident at all angles with the counter-axis were counted with equal efficiency.

Measurement.—To ensure that the CaF_2-crystal may be set at a fixed position as near to the counter-window as possible, the arrangement shown in Fig. 1 was adopted.

A metal cap C was so selected that it exactly fitted to the lower part of the counter-tube T. The CaF$_2$-crystal K was fixed to the centre of the cap by means of a thin, even layer of picien. A circular brass-ring R having almost the same thickness as the crystal, and external diameter equal to the inner diameter of C was also fixed inside the latter by a little picien. Now on sliding C over the counter-tube the upper surface of the crystal could almost be made to touch the mica window, and yet the crystal would not go further up due to the presence of the metal ring R and injure the mica window. A fixed position of the crystal was now selected by making a mark on C and a corresponding mark on T.

By placing the crystal within a quarter of a millimeter from the mica window in this way a maximum intensity of $\sim$158 counts per minute

FIG. 1. P—the point of the counter T; M—the mica window; R—a circular brass-ring 36 mm. in diameter, 0·5 mm. thick, 0·5 mm. broad; C—a metal cap of inner diameter 36 mm.; and K—the CaF$_2$-crystal.

was recorded. The zero-effect of the counter was about 8 per minute on the average. By repeated measurements it was seen that the activity shown by the crystal remained practically constant (within statistical error) for the first few days after the irradiation was stopped. To measure this long life activity it was therefore necessary to take readings at the interval of at least 15 days. But the difficulty in this was to ensure that the efficiency of the counting system may remain constant over the entire period of measurements, so that the readings taken at different times at the interval of 15 days or so can be compared with each other. To check this constancy, a standard source of RaD (which gives a very long life β-activity) was prepared; it was mounted inside a metal capsule in such a way that a thin linear bundle of β-rays was available. Each time, before the measurement with the CaF$_2$ activity was made, it was made sure that the counter gave almost constant readings when the RaD source was placed at a fixed distance from it. The activity from the CaF$_2$ crystal has been followed for 4 months up till now and it is being pursued further. The results obtained are described in §3.

(c) *The maximum energy of the Na^{22}-positrons from their Absorbability in Aluminium.*—The absorbability of the positrons emitted from Na^{22} was next measured by means of the point-counter. The CaF$_2$-crystal on the base of the metal cap C (Fig. 1) was placed in this case at a distance of $\sim$2·5 mm. from the mica window of the counter. The relative positions being marked once for all, thin sheets of aluminium beginning from 0·1 mm. thickness and
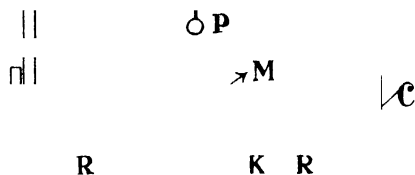
at intervals of 0·1 or 0·2 mm. were interposed between the crystal and the counter. As the Al-sheets were cut to the size of the metal cap, they could be directly put inside the cap and the latter slid over to the counter-bottom up to the fixed mark. The counter-reading was thus taken with each thickness of the Al-absorber, the maximum thickness employed being 1·4 mm. Al.

As in the case of measuring the half-life of Na^{22}-activity, here also the point-counter possesses some natural advantages over a tube-counter. The source of radioactivity (CaF_2-crystal) in these measurements is very small. Therefore the entire radiation from it can be practically covered by choosing a counter-opening of suitable diameter and placing the crystal very close to the opening. In measuring the absorbability of the radiation by interposing thin sheets of Aluminium between the crystal and the counter, the point-counter with the above specifications has the additional advantage that the beam of positrons entering the counter is confined to a narrow pencil, so that the thickness of the absorbing material traversed by each positron in the beam is very approximately the same. But as can be easily imagined, this is not the case in a tube-counter, where correction should be made on account of the extended effective surface of the counter. Besides, the small zero-effect of the point-counter is always a great advantage in measuring weak radiations as in the present case.

§3. RESULTS

(a) *The half-life of Na^{22}*.—The decay-curve of radioactive Na^{22} observed over a period of nearly 100 days is shown by the middle curve of Fig. 2. The abscissa represents time in days and the ordinate the positron intensity observed in 20 minutes. Each point has been observed for 20 minutes. The maximum positron intensity observed in the beginning was 152 per minute. The zero-effect of the counter was 9·8 per minute on the average. One possible source of error in the observed activity lies in the fact that it might be partly due to any contamination of the CaF_2 crystal from the slight traces of RaD (which would give a β-activity of nearly 25 years half-life) present in the Po-source. This possibility is, however, excluded on account of several reasons. During the irradiation of the crystal by the Po-source direct contact between the crystal and the source was carefully avoided by interposing a very thin gold-foil between the two, and the foil was carefully removed before measurement. Again, the observed activity has a half-life much less than 25 years which is approximately the half-life of RaD. Moreover, it is well known that the β-rays emitted from RaD are extremely soft, their maximum energy being ∼0·035 MeV, and they should be completely cut up by an Al-foil 0·01 mm. thick. On the other hand, it will be shown presently that the β-rays observed in the present case have a maximum range of ∼0·9 mm. of Aluminium and a maximum energy nearly 20 times as large as that of the RaD-β-rays.

The logarithmic plot of the decay-curve for Na^{22} is given by the lowest curve of Fig. 1, while the uppermost curve represents the same plot 10 times

magnified along the ordinate-axis. All the three curves have the common abscissa, and their ordinates differ only in scale. The half-life of the Na²²-
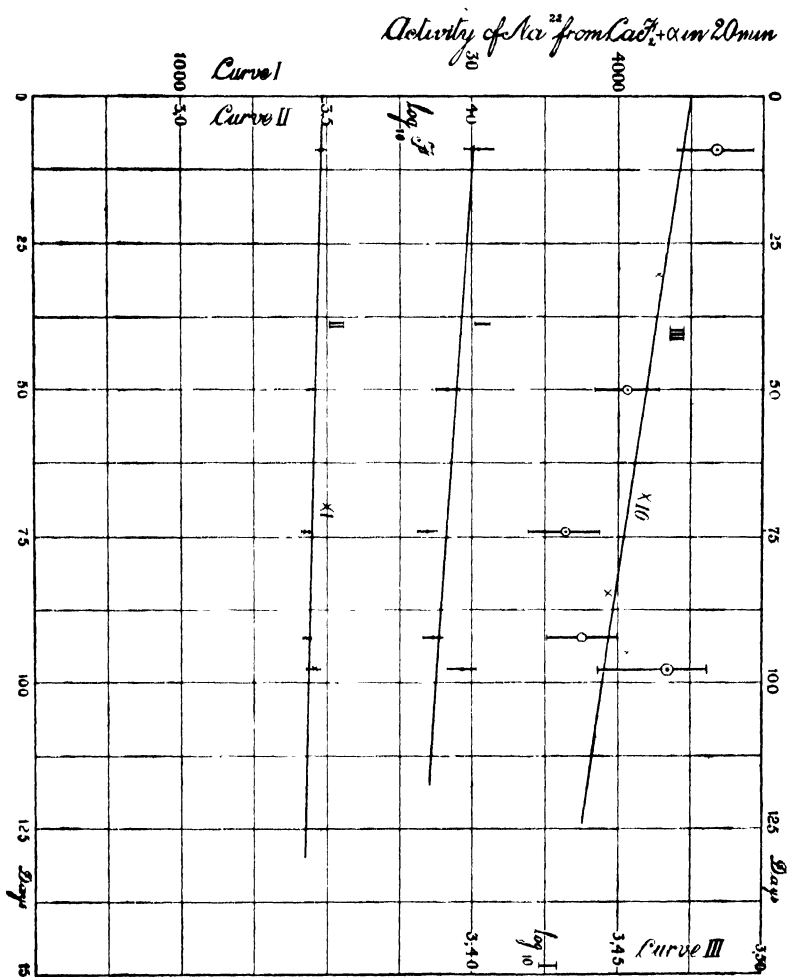
FIG. 2. I. The decay-curve of Na²².
II. The same as curve I on a log scale.
III. The same as II with the ordinate scale 10 times magnified.

activity extrapolated from the uppermost (magnified logarithmic) curve comes out to be 2·8 years with a probable error of ±0·5 years.

(b) *The absorbability of the Na²²-positrons.*—The absorption-curve of the positrons emitted from Na²² is shown in Fig. 3 in which the absorber thickness of Al in millimetres is plotted along the abscissa and the intensity of the transmitted positrons along the ordinate. Each point has been measured for 20 minutes. The curve has been followed up to an absorber thickness of

1·4 mm. Al.   The corresponding curve on a logarithmic ordinate scale is shown
in Fig. 4 which is approximately linear except towards the end.   An inspection
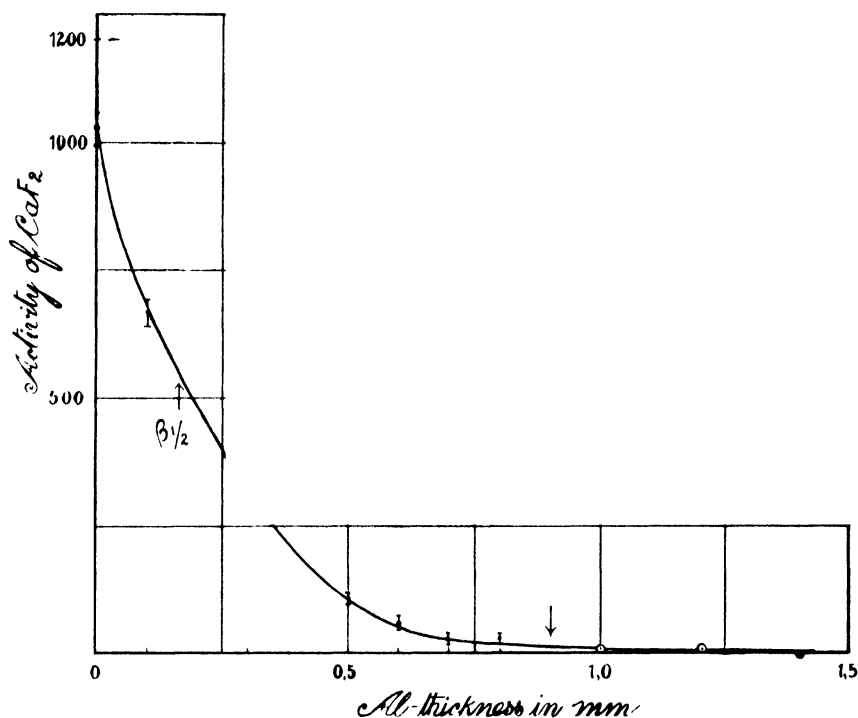
FIG. 3.   The absorption-curve for the β-rays from Na²².

of the absorption-curve of Fig. 3 gives a value ∼0·9 mm. of Al as the positron
range and a half-value thickness of ∼0·04 gm./cm.² of Al.   The half-value
thickness given by Frisch[2] is ∼0·03 gm./cm.² of Al, so that the agreement
with our observation is good.

The maximum positron-energy corresponding to the above range can be
obtained from the extrapolation-formula given by N. Feather[8].   It comes out
to be 0·6 MeV with a probable error of ±0·05 MeV in the range determination.
It was thought, however, advisable to check the extrapolation formula by
drawing a range-energy curve for β-rays using the most recent data[9] available.

These range-energy curves for β-rays drawn for two different ranges of
β-energy are shown in Fig. 5.   The β-range in gm./cm.² of Al is plotted along
the X-axis and the corresponding β-energy along the Y-axis.   The curve in
Fig. 5 gives the range-energy relation up to a β-energy of 3·1 MeV and that in
the upper curve in the low energy region from 0 to 0·08 MeV.

The maximum energy of the Na²²-positrons extrapolated from the above
range-energy curves comes out to be 0·61±0·05 MeV.

It is interesting to note that the range-energy relation for the β-rays as shown in Fig. 5 (and also given by Feather) are very nearly linear except in
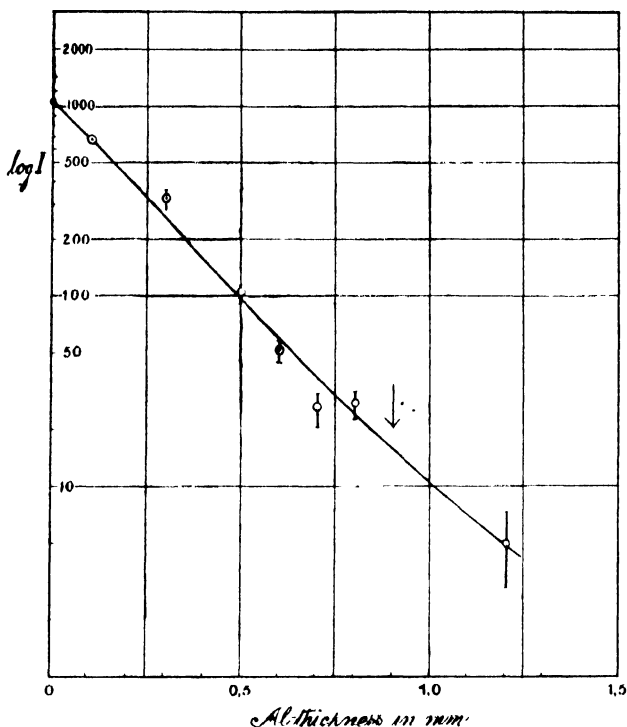
FIG. 4.   The logarithmic absorption-curve corresponding to Fig. 3.

the region of very low energies below 0·03 MeV.   A theoretical interpretation of this exceptionally simple relation between the range and the energy of the β-rays deserves investigation.

§4.   DISCUSSION OF RESULTS

According to our measurements the half-life of Na^{22} is 2·8±0·5 years and the maximum energy of the positrons emitted from Na^{22} is 0·61±0·05 MeV. This value for the half-life is much larger than the first approximate estimate by Frisch [2] (>6 months), but it agrees substantially with the value (3·0±0·3 years) obtained by Laslett [3] who produced Na^{22} by an entirely different process, namely by bombarding magnesium with deuterons of 5·2 MeV energy according to the reaction Mg^{24} (d, α) Na^{22}.   The observed limit of the positron-spectrum is independent of any theory and also agrees well with that given by Laslett (0·58±0·03 MeV).

The maximum energy of the positrons and the half-life of Na^{22} being known, they can now be plotted on the well-known Sargent diagrams for the

radioelements obtained by artificial disintegration process. Such a plot shows that Na^{22} falls approximately on the third Sargent curve indicating a nuclear spin-change $\triangle i = 2$ for the transition $Na^{22} \rightarrow Ne^{22} + e^+$. Two other

FIG. 5.   The range-energy extrapolation curves for β-rays.

nuclei which are known to fall definitely on the third Sargent curve are $_{15}P^{32}$ (14 d; 1·8 MeV) and $_{19}K^{42}$ (16 hr.; 3·5 MeV), both of which are emitters of negative electron. The large spin-change involved in these nuclear

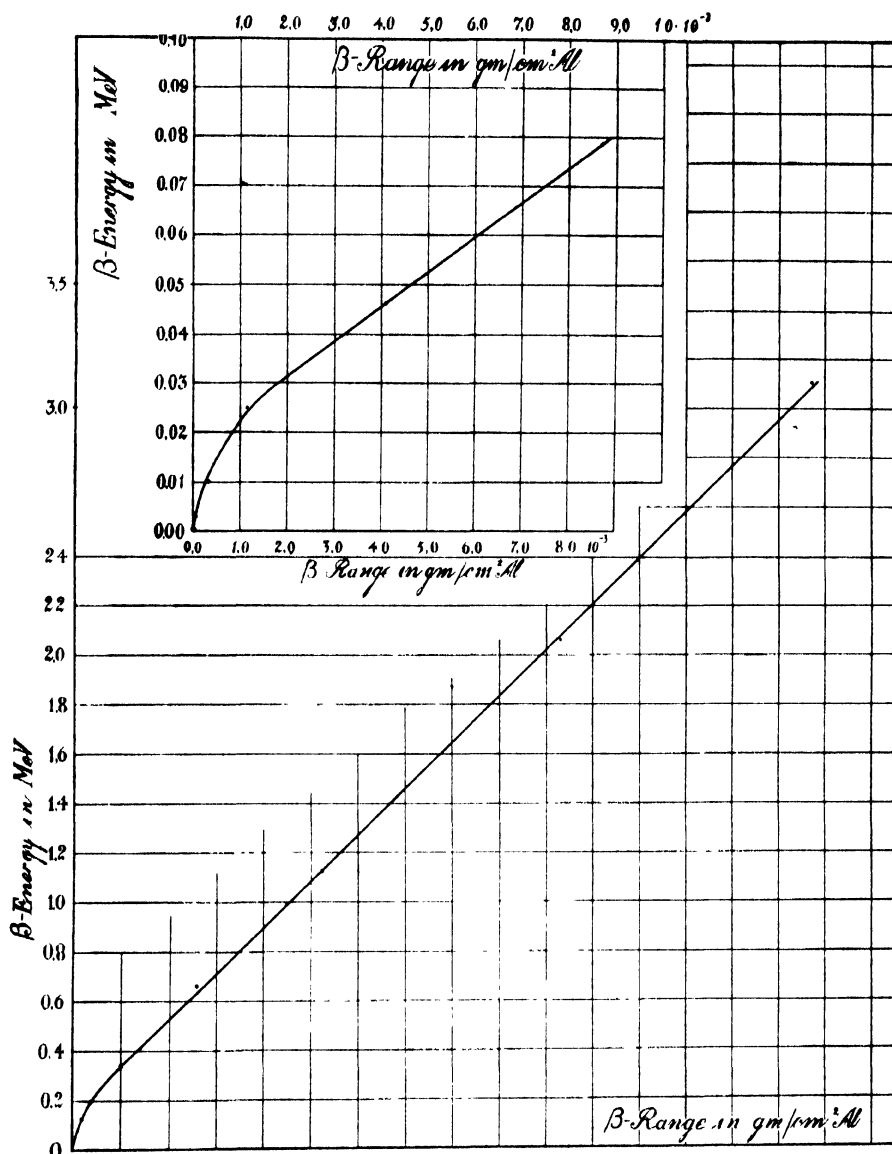transitions diminishes the probability of β-emission from these nuclei, although the transitions are not as forbidden as in the naturally occurring Rb^{86} ($\tau = 4\cdot3 \cdot 10^{11}$ yr.) and K^{40} ($\tau = 1\cdot5 \cdot 10^{13}$ yr.) for which the nuclear spin-changes involved in the β-emissions probably lie between 4 and 7.

Mass of Na^{22}.—With the help of our observed energy-limit of the positron spectrum a tentative calculation of the mass of Na^{22} can be made. The complete energy-equation for the disintegration of Na^{22} into Ne^{22} with the emission of a positron is

$$_{11}\text{Na}^{22} \rightarrow {}_{10}\text{Ne}^{22} + \text{e}^+ + \epsilon_0 + \text{e}^- \ (+\gamma),$$
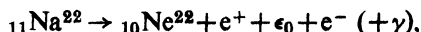
where ϵ_0 denotes the maximum kinetic energy of the positrons. An electronic mass e$^-$ is to be added to the right side in order to equalize the number of extranuclear electrons on both sides. The possibility of emission of any γ-radiation in the process is denoted by $(+\gamma)$. As we are not in a position to take the last into account, we shall get from this relation only a lower limit to the mass of Na^{22}. With $\epsilon_0 \cong 0\cdot61$ MeV and Ne$^{22} = 21\cdot9985$ (Pollard), we obtain Na$^{22} = 22\cdot00025$.

K-electron capture in Na^{22}.—The extremely long life positron emitter Na^{22} raises the important question of the possibility of capture of an orbital K-electron and emission of a neutrino by this nucleus as an alternative process to the positron emission. With Na$^{22} >$ Ne^{22}, the energy-condition for the K-capture

$$_{11}{}^*\text{Na}^{22} + \text{e}^- > {}_{10}{}^*\text{Ne}^{22} + \nu^0$$
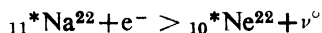
is evidently satisfied. (Here the asterisk denotes the nuclear mass and ν^0 is the mass of the neutrino.) According to the Fermi-theory of β-disintegration the probability of the K-capture process increases with increase in the nuclear spin-change occurring in the positron-emission. The probability has been calculated by Lamb [10] using older data, and according to him the probability for the K-electron capture comes out ~ 30 times as large as that for the positron emission. As such a large probability for the K-capture process appears physically unlikely, a recalculation on the Fermi-theory using $\Delta i = 2$ should be undertaken.

The physical consequence of K-capture in Na^{22} would be the decay of the Na^{22}-nuclei without any observable radioactivity and a gradual accumulation of Ne^{22}. Knowing the absolute yield of the positron-emission from Na^{22} from our experiment (see below), the probability of the K-capture could be experimentally determined if we could make an independent determination of the neutron-yield in the process (1) of §1. Unfortunately no direct determination of the neutron-yield is yet experimentally possible. Another plausible method would be the determination of the absolute intensity of the K-radiation accompanying the emission of the positrons from Na^{22}. The extreme softness and very low intensity of the K-quanta of Na^{22} expected, however, present experimental difficulties in this.

The absolute positron-yield from Na^{22}.—Finally, it is necessary to calculate the absolute positron-yield from Na^{22} from the intensity of the positrons extrapolated to zero-time. From the curves of Fig. 2 this intensity is $I_0 = 2985$ in 20 minutes. The initial strength of the Po-source was $P_0 = 0.4$ mC and the period of irradiation was 50 days. The mean strength of the polonium source averaged over this period is clearly

$$\bar{P} = \frac{1}{t_0} \int_0^{t_0} P_0 e^{-0.693 \frac{t}{\tau}} = 0.35_2 \text{ mC},$$

where τ = the half-life of polonium and $t_0 = 50$ days. Since 1 mC of polonium emits $3.6 . 10^7$ α-particles per second over a solid angle 4π the total number of α-particles falling on the crystal in 50 days is

$$\eta_\alpha = \bar{P} . 3.6 . 10^7 . 0.5 . 50 . 24 . 60 . 60 = 0.274 . 10^{14}.$$

The factor 0.5 comes from the fact that only one side of the Ag-disc contains Po-deposit.

The total number of positrons emitted by Na^{22} is now to be calculated. To do this the observed positron-intensity I_0 is to be increased by several factors: (*a*) As the active crystal is situated at a distance of at least 0.25 mm. from the counter-window, the fraction of the total number of positrons entering the counter is $\frac{1}{2} (1 - \cos \theta)$, where 2θ is the supplement of the angle subtended by a diameter of the counter-window at the centre of the crystal. (*b*) As there is one Ca-atom for every two F-atoms present in the CaF_2-crystal, a part of the α-particles which hit the Ca-atoms is being ineffective. The actual factor by which the positron intensity would be increased if the Ca-atoms in the crystal were all replaced by F-atoms can be calculated from the relative stopping powers of the F and the Ca-atoms and comes out to be 1.77. (*c*) A fraction of the positrons has been absorbed by the mica window of the counter. This latter had a thickness of 4.7 cm. air equivalent, which is equal to ~ 0.03 mm. Al. From the absorption curve of Fig. 3 the positron loss due to this thickness of Al is $\sim 12\%$.

If I = the number of positrons emitted per second corrected for the above three factors and λ = the decay constant of Na^{22}, the total number of positrons emitted by Na^{22}-nuclei if the latter were allowed to decay completely is evidently $n_\beta = I/\lambda$. Since the half-life of Na^{22} is $T \sim 2.8$ years, $\lambda = \ln2/T = 0.80 . 10^{-8}$ sec^{-1}. Hence finally

$$n_\beta = I/\lambda = 2.3 . 10^8$$

Therefore the absolute positron-yield from Na^{22} is

$$\frac{n_\beta}{n_a} \cong 8 . 10^{-6} \text{ per α-particle of Po.}$$

As no independent measurement of the neutron-yield from the F^{19} (α, n) Na^{22} is possible, the above result also gives us approximately the absolute neutron-yield from this process. Indeed $8 . 10^{-6}$ per α-particle of Po should be regarded only as a lower limit to the neutron-yield, for a fraction of the Na^{22}-neuclei formed in the reaction must have decayed without positron emission, by the capture of the orbital K-electron of sodium atom. We can therefore put the absolute neutron-yield of this reaction as $> 8 . 10^{-6}$ per Po-α-particle.

Although no significance should be attached to the exact numerical value of the neutron-yield calculated above, yet it shows that it is at least of the same order of magnitude as, if not greater than, the proton-yield of the reaction F^{19} (α, p) Ne^{22}. The latter has been determined rather accurately by Schintlmeister and Stetter[11] to be $\sim 3.6 . 10^{-6}$ per α-particle. The above result for the neutron-yield is very significant and very probably has its origin in the fact that for the excitation energy of 5·3 MeV, the maximum energy of the Po α-particles, the intermediate nucleus Na^{23} has got a higher number of excited states for the neutron-emission than for the proton-emission, and that the neutron-emission by resonance begins at a lower energy of excitation of the nucleus than the proton-emission. From a study of the excitation function of different branched reactions Wilhelmy[12], Maurer[13], Saha[1], Fünfer[14], Szalay[15] and others have shown that it is almost a general rule that those resonance levels of the intermediate nucleus which lead to the emission of neutrons or are formed by the capture of neutrons are more numerous and lie closer to each other than the resonance levels of the same nucleus which are associated with the emission or capture of charged particles. In the case of the branched reactions F^{19} (α, p) Ne^{22} and F^{19} (α, n) Na^{22} this fact has been directly established by Saha and it seems to have an important influence on the absolute yields of the two processes involved.

There are instances where the difference in absolute yield observed for individual nuclear processes of a branched reaction is to be ascribed to other causes, such as, difference in the energy-balance of the two branched reactions. For example, Szalay has shown that for the branched reaction:

$$_{13}Al^{27} (\alpha, n) \,_{15}P^{30}$$

$$(\alpha, p) \,_{14}Si^{30}$$
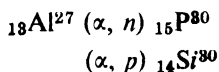
the absolute yield of the (α, p)-process is 6·9 times higher than that of the (α, n)-process for an energy of the exciting α-particle of 5·3 MeV. Both the (α, n) and the (α, p) processes set in at an α-energy of about 4 MeV. There are three resonance excited energy-levels of the intermediate nucleus $_{15}P^{31}$ common to both the processes, while two other levels for the proton-emission process probably exist which lie close to two of the three common levels. The energy-balance of reaction for the two processes, on the contrary, differs very widely, it being -3 MeV for the n-process and $+2.3$ MeV for the p-process, and this difference is believed to be the cause of the difference in the proton- and the neutron-yields observed.

In the case of $(F^{19}+\alpha)$ also the energies of the α-particles at which the (α, n)- and the (α, p)-processes set in are nearly equal and the energy balance of the neutron-process is less than that of the proton-process, though the difference is not so much as in the case of $(Al^{27}+\alpha)$. But quite unlike the case of Al^{27} the absolute yield of the $F(\alpha, n)$-process is found to be at least of the same order as, if not higher than, that of the $F(\alpha, p)$-process. The reason for this seems to be that there exist in this case at least twice as many resonance energy-levels for the n-emission process as those for the p-emission; out of these, three of the n-resonance levels lie near about the three p-resonance levels, but the other three n-resonance levels lie rather far from these common levels. Moreover, the first resonance level for the p-emission lies at a higher excitation energy than the first resonance level for the n-emission; so that when all possible excitation energies of the α-particles from zero to maximum are present, as is always the case in experiments with thick target, substantial neutron-yield sets in at quite a low energy of excitation of the nucleus, but no proton-emission practically takes place near about that energy. And further up above a certain minimum energy (sufficient to excite both the n- and the p-reaction), the intermediate nucleus Na^{23} has more modes of excitation which favour a neutron-emission from it than the corresponding modes for a proton-emission.

Summary

1. A Polonium source of α-particles has been prepared from some spent radon tubes by the method of purely chemical deposition with chemical purification.

2. Radioactive Na^{22} has been produced by irradiating a Fluorspar crystal by means of the Po-α-particles for 50 days. The half-life of the activity of Na^{22} has been measured by a point-counter with proper precautions for checking the constancy of its counting property over the long period of measurements. The half-life comes out to be $2 \cdot 8 \pm 0 \cdot 5$ years.

3. The maximum energy of the positrons emitted from Na^{22} has been determined by measuring their absorbability in thin foils of Al. The maximum energy comes out to be $0 \cdot 61 \pm 0 \cdot 03$ MeV. A plot of the Sargent curve for Na^{22} shows that a spin change of $\Delta l = 2$ is involved in the nuclear transition $_{11}Na^{22} \rightarrow {}_{10}Ne^{22}$.

The importance of this finding in relation to the probability of capture of a K-electron in Na^{22} is discussed.

4. A lower limit of the mass of Na^{22} is found from the observed maximum kinetic energy of the emitted positron. The value obtained is $Na^{22} = 22 \cdot 00025$.

5. The absolute positron-yield of Na^{22} is calculated and its significance in relation to the branched reactions $F^{19}(\alpha, p)Ne^{22}$ and $F^{19}(\alpha, n)Na^{22}$ is discussed.

REFERENCES

1 N. K. Saha.—Zs. f. phys., 110, 473, 1938.

2 O. Frisch.—Nature, 136, 220, 1935.

3 L. J. Laslett.—Phys. Rev., 52, 529, 1937.

4 N. K. Saha.—Trans. Bose Institute, 13, 159, 1939; Naturwiss., 27, 786, 1939.

5 L. R. Hafstad.—Journ. Frankl. Inst., 221, 191, 1936.

6 I. Curie.—J. Chim. Phys., 22, 471, 1925.

7 For details see Radioaktivität, St. Meyer u. E. Schweidler (1927), S. 279

8 N. Feather.—Phys. Rev., 35, 1559, 1930.

9 Madame P. Curie.—Radioactivité (1935), p. 294.

10 W. E. Lamb.—Phys. Rev., 50, 388, 1936.

11 J. Schintlmeister u. G. stetter.—Wien. Ber., 143, 608, 1934.

12 E. Wilhelmy.—Zs. f. phys., 107, 746, 1937.

13 W. Maurer.—Zs. f. phys., 107, 721, 1937.

14 E. Fünfer.—Phys. Zs., 18, 541, 1937.

15 A. Szalay.—Zs. f. phys., 112, 29, 1939.

V. EFFECT OF HIGH CONCENTRATION AUXIN ON THE GROWTH AND ROOT FORMATION IN IMPATIENS

By B. Dutt and A. Guha-Thakurta

In a previous communication [1] it has been shown that like the root forming substance, described by Cooper,[2] the naturally occurring growth substance in the coleoptile of Triticum is also attracted by the high concentration auxin towards the region of its application and the growth changes in the organ is directly related to the concentration of the internally contained hormone at the applied region. Now the question arises whether the same substance in the plant is responsible for both the activities of root formation and growth promotion or are there specifically different substances?

Bouillenne and Went [3] have suggested that a special substance in the plant is associated with root formation and they named it rhizocaline. They were supported by Nemec [4] who showed that buds or cotyledons not only store food materials but also special root forming substances or rhizogenes.

On the other hand the works of other workers, Thimann and Koepfli,[5] Kogl,[6] etc., provide the proof that the root forming and the growth promoting substances are identical with each other.

Went [7] in a recent paper, however, suggests that the existence of some specific hormones in the plant, in the form of calines, are separately responsible for the root, shoot and leaf growth. According to him caulocaline, coming from the root, induces the growth of the stem, rhizocaline, from the cotyledon or leaf, induces the growth of the root and phylocaline, formed in leaf and cotyledon, is responsible for the growth of the leaf. The calines, according to him, are reactive in conjunction with auxin.

Fischnich [8,9] has shown that the callus of Populus nigra may produce roots or shoots depending upon the concentration of auxin. A high auxin content favours root formation. But the root forming callus can be induced to form shoot by reducing its auxin content. From the works of Fischnich it becomes difficult to conceive of a separate identity of the root and shoot forming hormones in plants.

In our present investigation the effects of high concentration auxin on both growth promotion and root formation have been observed in the same organ—the stem of Impatiens. The reactions were studied at different regions of the stem and under various morphological conditions of the plant. The data obtained might possibly be of some help toward the interpretation of the causes of different reactions: whether specifically different substances are the causes of the different reactions or the same substance is the potent

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factor underlying both the reactions; the difference in reaction being only the effect of difference in concentration of the same substance or it might be brought about by the presence of other chemical substances or influenced by the physiological condition of the tissues.

Our observation included the estimation of the carbohydrates of the different treated and untreated regions of the stem, at definite periods of the experiments, and also the study of the histology of the formation of root.

<div align="center">PROCEDURE</div>

Potted seedlings of Impatiens of the same age and approximately of same height were used in the different experiments. In observing the effect of high concentration auxin, 1 % Indole acetic acid in lanoline paste, was used.

It was observed from some preliminary experiments that indole acetic acid paste of the above concentration produces localized roots in Impatiens very readily. Impatiens grows very vigorously during the rains, so this season was selected for the experiments.

The experiment was started by observing the effect of auxin, either separately or simultaneously, at different regions of both intact and decapitated plants. Experiments were also conducted on detipped and completely defoliated specimens.

The auxin paste was applied round the stem in a ring to the extent of a centimeter. In control plant only pure lanoline was applied in the same way.

The growth increase and the number of roots after they came out were individually recorded, for each plant, every twenty-four hours, up to the tenth day from the commencement of each set of experiment.

In estimating the carbohydrates, the reducing sugars and the total carbohydrate were separately estimated from similar specimens. Three sets of plants treated at the apical, central and the basal regions were separately handled and of each of these sets the carbohydrates in all the three regions, apical, central and basal, were separately estimated out. The carbohydrate and sugar were estimated 2, 4 and 6 days after the application of auxin.

In studying the histology of root formation, sections were made through the treated regions after 2, 4 and 6 days of the application. Photo-micrographs of the sections were taken to show the initiation and gradual development of the root and the tissue from which it has developed. The sections of the different regions of the control plant were also examined to study the distribution of the vascular bundles.

<div align="center">EFFECT OF HIGH CONCENTRATION AUXIN ON THE GROWTH OF IMPATIENS</div>

It has been previously mentioned that the growth measurement and the root counting were made on the same plant. Two sets of experiments were run side by side; one with intact plants and the other with top-cut plants. In both the sets the plants were divided equally into different groups each of

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which was treated with auxin at one particular region of the stem. Equal number of control experiments were also made in which instead of auxin paste, pure lanoline was applied at the corresponding regions.

In measuring growth, only the length of the stem from above the ground to the base of the apical bud was taken. The lengths were measured every twenty-four hours till the tenth day of the experiment. The percentage of growth increase in relation to the original length, were calculated out from the diurnal average growth of each group of plants.

In the intact plant the effects of the application of auxin were investigated in the following regions of the stem: (1) Apical—just below the apical bud, (2) Central, (3) Basal—just above the ground, (4) in two places in the upper part of the stem—one just below the apical bud and the other in the mid-region below the whorl of leaves. The percentages of growth increase of intact plant, under varying conditions of treatment, calculated from the average diurnal growth of each group, are graphically represented in figure 1. The corresponding growth curve of the intact control plant has also been included in the same figure for comparison.
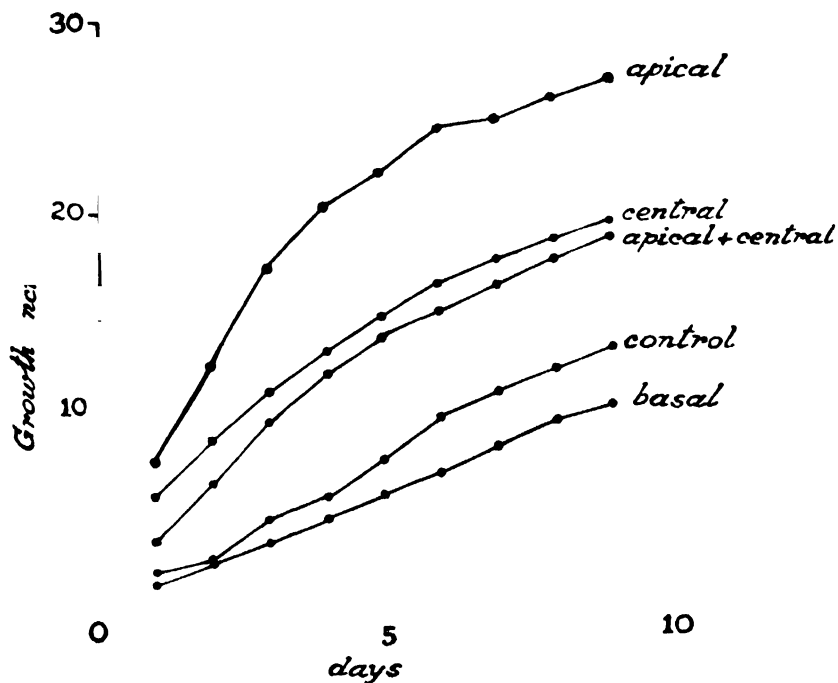
FIG. 1. Comparative growth increase of Impatiens (intact plant) by treatment with auxin at different regions of the stem.

The top-cut plants were treated in the following way: (1) Apical—at the bare upper end of the stem, (2) in the central region below the whorl of leaves,

(3) in both the regions in the same plant. Percentages of the growth increase of the different groups of experiments were calculated from the average diurnal growth of each group. The growth curves of detipped specimens under different conditions of treatment along with that of the control plants are graphically represented in figure 2.
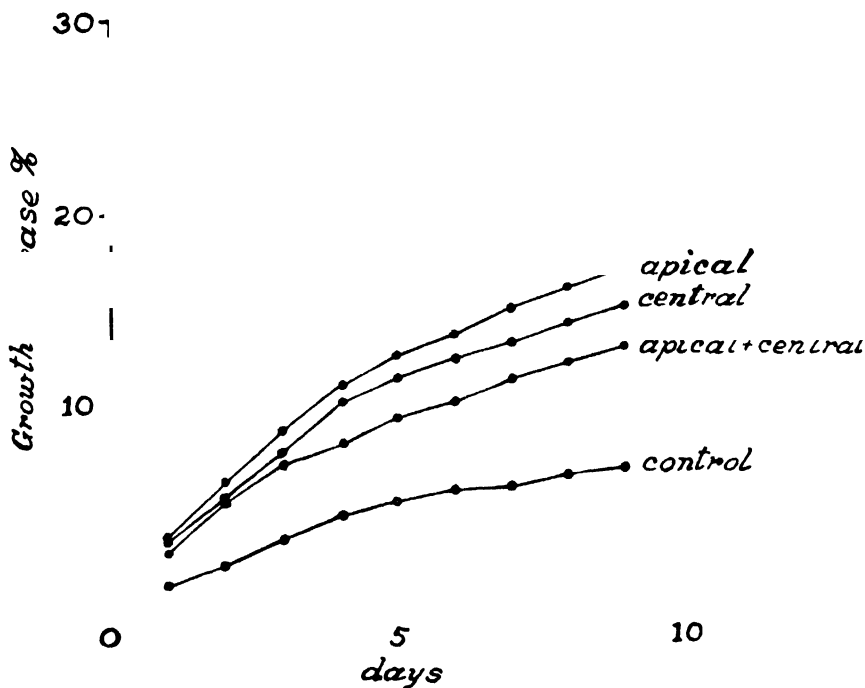
FIG. 2. Comparative growth increase of Impatiens (decapitated plant) by treatment with auxin at different regions of the stem.

It will be found from comparison of the curves in figure 1 that the apically treated plant grows most vigorously within the period of our observation. Next comes the centrally treated plant, while the basally treated plant grows less even than the control. The plant which was treated in the two places in the upper region, apical and central, grows less even than the centrally treated plant, in spite of one of the points of application being the apical end of the stem. The apically treated plant grows most vigorously within the first three or four days of the application of auxin; the vigor of growth is much reduced after this period. In the centrally treated plant also the daily average growth is comparatively greater in the first four or five days of the application of auxin, than that during the rest of the period.

In the top-cut specimens also apical application of auxin is seen to have induced the greatest elongation of the stem. When auxin was applied in apical and mid-regions simultaneously the growth was less than that of the

centrally treated plant as was found in the intact plant. That the removal of the top greatly inhibits the longitudinal growth of the stem will be seen from a comparison of the growth of the detipped controls with those of the intact controls.

It was further noted that the axilary buds readily developed as a result of the removal of the top and the top-cut control plant became bushy within the period of observation. But in the decapitated plant which were treated with auxin, the axilary buds did not develop so readily and there was no appreciable difference between them and the intact plant on this point.

A group of decapitated plants were completely defoliated; some of these were treated apically with auxin paste and an equal number with pure lanoline. The auxin treated plant increased in length by 1·25% on the second day of treatment. On the third day the tips of more than half the number of auxin treated plants became limp and crumpled and the rest though not crumpled did not increase in length and in some cases even decreased from the previous day's lengths. In four or five days all the auxin treated plants became crumpled and bent to the ground. The defoliated controls which were apically treated with pure lanoline increased by 0·64% on the second day. On the third day the growth was totally stopped in all the specimens, some even shrinking in length. The process of decay was more slow in the control than in the auxin treated plants. Some of the control plants survived decay within the period of our observation, though not increasing in length. None of them, however, survived complete disintegration beyond a fortnight.

Effect of High Concentration Auxin on the Root Formation in Impatiens

Observation on the effect of auxin on the root formation at different regions of Impatiens stem was made from the specimens which were used in recording the effect on growth given in previous chapter. It should be mentioned here that the auxin treated plants only produced roots at the region of treatment and no adventitious root was formed in the control plant. In the treated plant the time of rooting and the number of roots formed depended on the region of application of auxin. In counting the number of roots, only the individual root heads which sufficiently protruded out of the epidermis of the stem, were taken into account. As the roots in a region did not all come out on the same day, the number of roots were recorded after their first appearance every day. The observation was continued till the tenth day of the application of auxin.

The number of roots in each plant of a group varied. Each individual plant was separately counted every day and the average number for each group was calculated out. Records of roots in all the experiments are given in the Tables I, II and III. Table I contains the average of three groups of

TABLE I

Root formation in Impatiens stem (intact plant) by treatment with high concentration auxin at different regions of the plant

Region treated	Number of roots formed (average of 50 plants)								
	Days after treatment								
	1	2	3	4	5	6	7	8	9
Apical region ..	..	..	..	0·4	6·4	16·8	21·4	23·2	24
Central region ..	..	..	..	..	2·9	10·1	17·8	21	22·7
Basal region ..	..	..	..	..	..	0·9	3·6	7·8	11·6

TABLE II

Root formation in Impatiens stem (decapitated plant) by treatment with high concentration auxin at different regions of the plant

Region treated	Number of roots formed per plant (average of 25 plants)								
	Days after treatment								
	1	2	3	4	5	6	7	8	9
Apical region ..	..	..	..	..	1·9	5·0	9·8	11·6	13
Central region ..	..	..	..	..	2·4	7·4	11·6	14·1	14·8

TABLE III

Root formation in Impatiens stem by simultaneous treatment of apical and central regions with high concentration auxin in intact and decapitated plants

Region treated		Number of roots per plant (average of 25 plants)								
		Days after treatment								
		1	2	3	4	5	6	7	8	9
Intact plant	Apical	..	..	..	..	0·2	0·8	1·4	1·5	1·8
	Central	..	..	..	..	1·4	3·9	5·8	6·5	6·8
Decapitated plant	Apical	..	..	..	..	0·08	0·2	0·6	0·88	1·28
	Central	..	..	..	..	0·28	1·44	3·04	3·32	4·24

intact plants treated apically, centrally and basally. Table II contains the records of two groups of decapitated plants, one treated at the apical stump and the other at the mid-region below the whorl of leaves. In Table III are given the records of two groups of plants, one intact and the other

decapitated, being treated with auxin simultaneously in the apical and central regions.

From an inspection of Table I, it will be found that root appears earliest in the apically treated plant, after four days of treatment. First appearance of the root in the centrally treated plant is after five days and that in the basally treated plant after six days. Regarding the average number of roots which appear within nine days, there is not much difference between the apically and centrally treated ones—it being slightly greater in the former. The difference is most marked when either of them is compared with the basally treated one, which has considerably smaller number of roots.

Some variation in the arrangement of roots was also observed in different treated regions. At the basal region roots when formed seemed to be arranged in longitudinal lines round the stem. In the mid-region too the lines of roots were quite distinguishable in the major portion round the stem, though the intervening spaces between the lines were not so marked as in the basal region. In the apical region the lines are not distinguishable except in a few places; in most parts round the stem they grow very closely to one another in a clump rather than in lines. Photographs of root formation in plants treated apically, centrally and basally are given in figure 3 (plate XIII).

It will be found from comparison from the data in Table II, with those in Table I, that the number of roots either at the apical region or at the middle region is much less in the decapitated plant than in the intact plant. In both the groups of the decapitated plants roots appeared on the same day, viz. five days after treatment. The number of roots in both the groups is almost equal, though slightly greater in that treated in the central region below the whorl of leaves.

It will appear from a comparison of Table III, with the other tables that when the apical and central regions are simultaneously treated in either intact or decapitated plants, the number of roots produced in both the treated regions put together is much less than in similar plants treated separately in either of the above regions. In both the intact and the decapitated plants the roots appeared simultaneously after five days in both the treated regions. In both of them the number of roots are comparatively greater in the centrally treated regions than in the apical ones.

In the decapitated plants which were completely defoliated apical treatment with auxin did not produce any root. It has been previously stated that they decay down within three or four days of the treatment of auxin.

ESTIMATION OF CARBOHYDRATES OF DIFFERENT TREATED AND UNTREATED REGIONS OF THE PLANT

It has been shown by some workers that the treatment with high concentration auxin induces a translocation of carbohydrates to the point of

treatment. Stuart[10] found that when the kidney bean cuttings were treated by immersing their bases in 0·01% indole acetic acid there was a directional shift of large amounts of nitrogen and carbohydrates from the leaves and cotyledons to other parts, principally to the treated part. Alexander[11] also found by applying 2% indole acetic acid on the apical cut surface of bean plant that a translocation occurs of carbohydrates to the point of treatment and he also found that the condition caused the simple carbohydrates to be condensed into complex polysaccharides.

In the present investigation carbohydrates of all the treated and untreated regions of differently treated plants were estimated at definite intervals of time to see how the carbohydrate contents of the different regions vary in relation to the time of treatment. Three sets of plants were treated apically, centrally and basally. Carbohydrates were estimated for the apical, central and basal regions separately of each of the sets. Carbohydrates of the three regions of the control plants were also investigated for comparison.

In these investigations, the reducing sugar and the total carbohydrate contents were separately estimated. In estimating reducing sugar the plant material was completely macerated and boiled in a closed bottle for an hour in 50% alcohol. After filtering, the extract was made alcohol free. The sugar was then estimated by Benedict's method.

In estimating total carbohydrates the crushed plant material was boiled in 20% hydrochloric acid in a closed bottle for half an hour and the filtrate was tested by Benedict's method for estimation of sugar. The total carbohydrate in this case, therefore, consists of reducing sugar and other acid hydrolysable carbohydrates.

Reducing sugars and total carbohydrates were estimated from different samples taken from similarly treated plants. For each of the two kinds of experiments ten pieces were taken from the same region of ten similarly treated plants. The lengths of the pieces were always equal, being two centimeters. Three regions of each plants contributed to three different experiments. Pieces were always cut out for experiments at 11–30 A.M. The outer surface of the pieces was made lanoline free by carefully rinsing with a brush soaked in xylol. The fresh weight of the pieces for each experiment was taken and the solution was made up to a particular strength following the method described above.

The estimations were made after two, four and six days of the application of auxin. The investigation was not further continued due to the inconvenience of handling the specimens in which the roots had grown sufficiently big by that time. When the roots had enlarged it became difficult to make the pieces perfectly lanoline free and in that condition considerable error might be introduced in taking the fresh weights of the materials.

Each solution was tested three times and the mean value was taken for estimating the sugar. Two complete sets of experiments were made and the mean values of the analytical data are diagrammatically represented in figure 4.
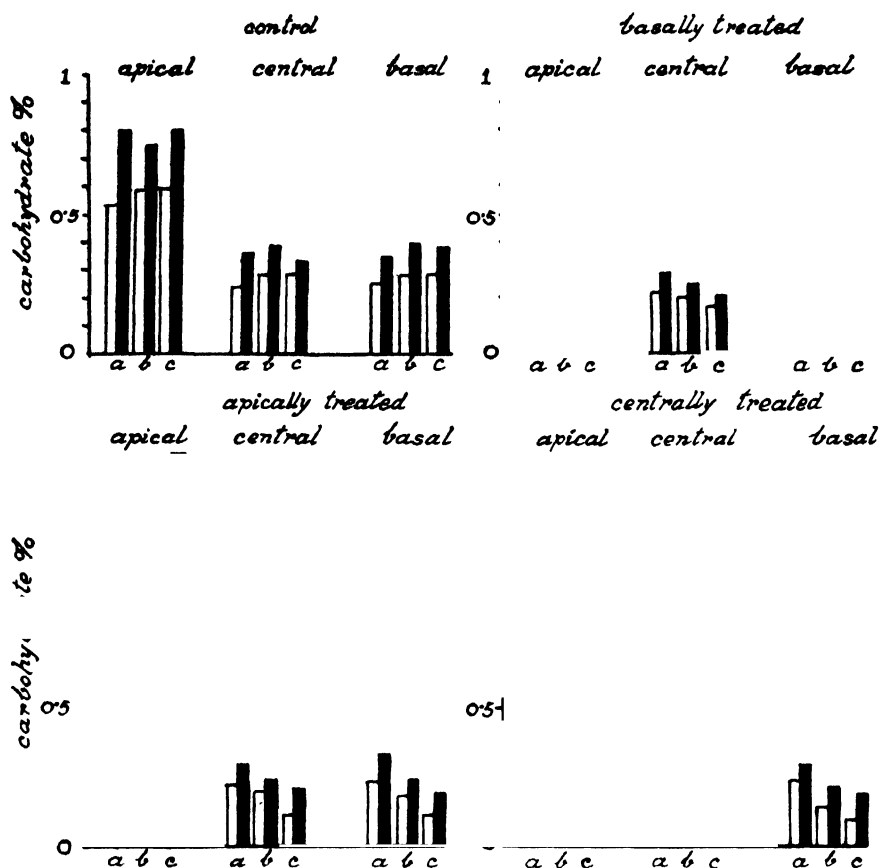
FIG. 4. Diagrammatic representation of the variation of carbohydrate content in the treated and untreated regions of Impatiens stem by treatment with high concentration auxin. White columns represent reducing sugar and dark columns represent total carbohydrate: (a) on the third day; (b) on the fifth day; (c) on the seventh day of the treatment.

From inspection of the results of the control plants (Fig. 4) it will appear that the apical region contains both reducing sugar and total carbohydrates in a comparatively greater amount than in any other regions of the stem. In the amounts found in the middle and the basal regions there is no marked difference. But if the ratios of the total carbohydrates and the reducing sugars of the different regions of the plant, given in Table IV, are compared they will be found more or less equal in all the regions. This indicates that the high value of the total carbohydrates in the apical region is mostly due to the presence of the high content of the reducing sugar in it.

In the treated regions the quantity of the total carbohydrates is greatly increased. The amount of the reducing sugar which comparatively increases after two days of the treatment than the corresponding amount of the control

TABLE IV

The ratio of total carbohydrate and reducing sugar in different regions of treated and untreated plants

Regions treated	Days after treatment	Apical	Central	Basal
Apical	2	1·4	1·2	1·4
	4	2·2	1·6	1·4
	6	4·0	2·0	1·6
Central	2	1·6	1·5	1·2
	4	1·7	2·2	1·6
	6	1·5	3·5	1·9
Basal	2	1·5	1·3	1·3
	4	1·4	1·2	2·2
	6	1·2	1·3	3·1
Nil (control)	2	1·5	1·3	1·4
	4	1·3	1 2	1·4
	6	1·4	1·2	1·3

plant, gradually declines on the subsequent days. In the untreated regions the quantities of both reducing sugars and total carbohydrates gradually decline.

In the treated, middle and basal regions the amounts of total carbohydrates when reach their maximum value six days after treatment, become more or less equal to that normally present in the apical region of the normal plant. The reducing sugar in them is noticeably less than that normally present in the apical region of the control plant and consequently when the ratios of total carbohydrate to the reducing sugar are compared the values for the treated regions are much higher than that in the apical region of the control plant.

In all the treated regions the ratio between the total carbohydrate and the reducing sugar is seen to increase gradually.

HISTOLOGICAL STUDY OF THE FORMATION OF ROOT IN AUXIN TREATED REGIONS OF IMPATIENS

Histological response of the application of heteroauxin has been investigated by different workers. Harrison [12] found that in Iresine lindinii the cells of the pericycle and its derivative rays and phloem were the most generally responsive and the roots developed from them, but neither the cambium nor the endodermis was markedly stimulated and no root was formed

from them. Hamner [13] found in Mirabilis jalapa that the pericycle and interfacicular cambium are the most responsive and roots developed from them and also from piths adjacent to the vascular bundles; the facicular tissues were unresponsive. Dorn [14] found that adventitious roots are formed from different tissues in different plants. According to him in Coleus hybridus, roots are formed from pericycle, in Solanum lycopersicum from the outer leptome parenchyma, in Passiflora quadrangularis and Plumbago ceylanica from interfacicular cambium, in Nasturtium officinale and Cochlearia officinalis from pericycle, interfacicular cambium or the margin of the facicular cambium and in Cochlearia armoracia from parenchymatous parts of pericycle bridging the space between two bundles of the stele.

In our present investigation on the histology of the formation of root in Impatiens it was found that the root develops only from the interfacicular cambium. The photo-micrographs of three sections taken after two, four, and six days through the treated region of the centrally treated plants are given in figures 5, 6 and 7 respectively.

Figure 5 (plate XIII) shows how the interfacicular cambium is slightly thickened at some places even after two days of the treatment, forming the rudiments of adventitious roots.

Figure 6 (plate XIV) represents a typical section after four days of treatment with auxin. In it two rudimentary roots are shown, one of them has fairly enlarged though yet embedded in the cortical tissue.

Figure 7 (plate XIV), representing a section after six days, shows two developed roots which have already protruded out of the epidermis.

When it was definite that the roots in the treated region developed from the interfacicular cambium in Impatiens the arrangements of the vascular bundles were studied in the different regions of the normal plant with the expectation of finding a clue for the modification in arrangement of roots in the different treated regions as previously mentioned. Photo-micrographs of the sections of the apical, central and the basal regions of the same plant are given in figs. 8, 9 and 10 (plate XV) respectively.

On counting the bundles it will be found that the apical section contains 18, the central 11 and the basal 6 in number. If traced from the apical to the basal region it will appear that two or three individual bundles in the apical region which are very closely situated to one another have coalesced together into one in the lower region. Consequently, the individual bundles in the basal region are larger than those in the apical region. With the reduction of the number of individual bundles in the lower regions the number of interfacicular spaces have also decreased. Also the apical stem is much thinner than in the basal region and has got the greatest number of interfacicular spaces for the growth of roots; further the intervening spaces between the roots in the apical region are occupied by very small bundles. These three factors have combined to make the roots grow so thickly in a clump in the apical region. While in the lower regions the stem is thicker, the interfacicular

spaces are comparatively fewer in number and the intervening spaces between the roots in the same plane occupied by big bundles, are greater. Consequently, the roots of these regions growing in a few interfacicular spaces, kept apart by the intervening big bundles, look like well differentiated lines.

DISCUSSION

In the present investigation both the activities of growth promotion and root formation induced by the application of auxin have been studied on the same plant. The modification of both of these activities as effected by subjecting the plant to various morphological conditions have also been simultaneously studied on the same plant. This experiment, therefore, enables us to study, both quantitatively and qualitatively, how the individual reactions are influenced by the various conditions of the plant. We shall now discuss whether the evidences obtained enable us to draw any tentative conclusion on the vexed question whether in the plant one or more than one specific substance exist, which are responsible for growth and root formation in it. In the intact plant growth activity reaches its maximum when the region of treatment is apical. When the region of treatment recedes down-ward the growth activity decreases till it becomes less even than that of the control when the region of treatment is the base. In our previous investigation on the effect of high concentration auxin on the growth of the coleoptile of Triticum,[1] it was concluded that the growth of the coleoptile is effected by the concentration of the internally contained growth substance at the region of treatment; apical treatment enhanced growth much more than in the normal plant because there is a greater concentration of the naturally occurring growth substance at the growing apical region and the tissue there was capable of elongation, while under basal application the growth was below normal because the growth substance was withdrawn from the growing apical region and concentrated in the basal region, where the tissue was not in a condition for further elongation. The present results of the effect of auxin on different regions of intact Impatiens plant are essentially the same as those of the coleoptile of Triticum. Hence the same conclusion seems to be quite plausible for the explanation of the causes of the modification of growth of Impatiens as effected by the treatment of auxin at its different regions.

Now let us see how the root formation has been effected by the application of auxin at the different regions and consider about the causes of the effect. We find that roots have formed only in the treated regions. That the treated auxin is not directly related with the formation of root and that it only brings about a concentration of the internal factor or factors which effect the formation of roots, have been agreed upon by many workers. There is disagreement only regarding the specificity of the root forming substance. Unlike the growth activity which is manifested only when the upper regions are treated with auxin the root forming activity is seen to be distributed throughout the entire

length of the Impatiens stem. This difference in behaviour between growth and root forming activities does not necessarily lead to the conclusion that they are due to the actions of different substances. This can be explained even if the same substance be associated with both the reactions. It has been previously mentioned that in apical application the high extensibility of the tissues together with the concentration of internal hormone produces enormous growth. In basal application the growth of the plant is reduced because the basal tissues are not capable of further elongation in spite of the concentration of hormone at this region and this leads to the reduction of the normal growth at the apical region due to the withdrawal of hormone from the same. It has been found that the roots come out in Impatiens from the interfacicular cambium (figs. 5–7). Concentration of hormone at the basal region, though incapable of producing further elongation of the region, can stimulate the interfacicular cambium and thereby produce roots in the region. The cause of the variation in the time of the first appearance and number of roots in the apical and basal regions can be attributed to any one or all of the following causes: The difference in the physiological condition of the tissues in the two regions, the number of interfacicular spaces, the concentration of internal hormone, and the concentration of other internal factors such as carbohydrates (fig. 4). The explanation may appear plausible, but it is premature at this stage of the experiment to conclude that the same substance is associated with both the reactions.

When the plant is decapitated both the activities under apical treatment are to a degree lessened in comparison with the similarly treated intact plant. This shows that the presence of the apical bud is invigorating to both the activities. It either supplies one particular substance to add to the vigour of both the activities or both the specific substances, if two are necessary for two different activities. When the decapitated plant is treated in the mid-region the growth becomes slightly less than the apically treated decapitated plant but the number of roots have been comparatively slightly greater. The smaller growth in the mid-region can reasonably be attributed to the limited stretching capacity of the tissues of the region, quite similar to what takes place in the case of centrally treated intact plant.

When both the apical and the central regions are treated in a plant the growth of the plant becomes less even than that of the centrally treated plant. It shows that the substance responsible for growth, being attracted to two regions could not be mustered in sufficient quantity in any one of the regions necessary for the induction of maximum elongation of that region. The total number of roots formed under the condition has also been comparatively much less than that in the plant treated at either of the regions. The explanation is applicable in this case too that it is due to the division of the substance, whether it might be specific for root formation or it might be the same substance which promotes also the growth, that prevents sufficient concentration in any region to produce a large number of roots. The

decapitated plant under such double treatment behaves in the same way in its growth and root forming activities, both becoming comparatively less vigorous than in the similar plant in which any of the regions has been treated separately. In both the intact and decapitated plants the number of roots, when treated simultaneously in two upper regions (Table III), have been much greater in the mid-region than in the apical region. This greater number in the mid-region signifies that it has been brought about by the greater concentration of either the growth substance or the specific root forming substance in the region.

In the completely defoliated stem apical application slightly enhances the growth in the first twenty-four hours. After that the stem instead of further increase rather decreases in length or decays in spite of the presence of the root which according to the suggestion of Went [7] is the source of caulocaline. No root was formed in the treated region, showing that defoliation affects both the growth and root formation alike. It is quite apparent that the slight increase in the first twenty-four hours was due to the concentration at the apical region of the existing growth substance in the stem.

From the above general survey of different experiments it appears that both the growth promoting and root forming activities are similarly effected by the morphological condition of the plant and the source of hormone supply whether they are specific or general, are the leaves and the apical bud, since the removal of them affects both the activities alike.

Total carbohydrates increase gradually at the treated region within the period of our observation. That the increase at the treated region occurs due to the directional shift from the untreated regions, will be evident from the gradual reduction of the same in those regions. The percentage of reducing sugar which increases at the beginning of the experiment as will be evident from the record after two days, gradually declines on subsequent days. The present result, therefore, supports the conclusion of Alexander,[11] that in the treated region the simple carbohydrates are condensed into complex polysaccharides.

It has been previously mentioned that in the treated central and basal regions the percentages of both the reducing sugar and total carbohydrates, when they reached their maximum, do not exceed even those present in the apical region of the control plant. But the ratios of the total carbohydrate to the reducing sugar are significantly increased in the treated basal and central regions in comparison with the apical region of the control plant. This indicates that the root formation is related not merely to the high carbohydrate content of the tissue but also to the high value of the ratio of the total carbohydrate and the reducing sugar.

The high value of the reducing sugar at the apical region of the control plant signifies its relation with growth of the plant. In the treated region the percentages of reducing sugar increase at the early stage of the treatment. The growth under apical treatment also greatly increases at the early stage of the treatment, gradually declining with the formation of root. The increase

of the percentage of the reducing sugar at the early stage may, therefore, have some influence on the increase of growth.

SUMMARY

In the present investigation the reactions of the effect of high concentration auxin on both growth and root formation were simultaneously studied in the stem of Impatiens in order to understand whether specifically different substances in the plant are the causes of the different reactions or the same substance is the potent factor underlying both the reactions. The reactions were studied at different regions of the stem and under varying morphological conditions of the plant. The study of the carbohydrate metabolism in the treated and untreated regions and the Histology of the formation of root in the treated region were also undertaken as an additional investigation.

In intact plant apical application of auxin induced the maximum growth of the plant within 9 days, the period of observation; in central application the growth was comparatively less than that of the apically treated one and in basal application the growth of the plant became less than even that of the control plant. When the apical and the central regions of the stem were simultaneously treated the growth was less than that of even the centrally treated plant.

The number of roots in the apically and centrally treated regions was almost equal; in the basally treated region the number was much less. When the apical and the central regions were simultaneously treated the total number of roots in both the regions was much less than when any of the regions was individually treated.

When decapitated the growth of the plant was greatly inhibited. Treatment with auxin at the apical or the central regions, under the condition, increased the growth almost equally. When both the regions were simultaneously treated together the growth was comparatively much less than when any of the regions was individually treated.

The number of roots in the apically and centrally treated decapitated plants was almost equal but when both the regions were treated simultaneously in a plant the total number of roots in both the treated regions was comparatively much less than when any of the regions was treated individually.

When the decapitated plant was completely defoliated the growth was very near nil and ultimately the plant decayed down. When such plants were apically treated with auxin there was slight increase of growth on the first day of treatment but disintegration followed more rapidly in them than the controls. No root was formed in the treated region under the condition.

In the normal plant the percentages of both reducing sugars and total carbohydrates were greatest at the apical region. In the central and basal regions there was not much difference in the percentages of any of them.

In the treated region the percentage of reducing sugar increased up to the third day of treatment with auxin; after that there was a gradual decline while

the percentages of total carbohydrates gradually increased in all the treated regions. The ratio of the total carbohydrate and the reducing sugar was progressively increased in the treated region. In the untreated region of the treated plant the percentages of both the reducing sugar and total carbohydrate fell down rapidly.

In the treated region the roots developed from the interfacicular cambium. The rudiments of roots were formed even on the third day of the treatment.

It has been concluded that both the growth promoting and the root forming activities, brought about by the application of high concentration auxin, are similarly effected by the morphological condition of the plant and that the source of hormone supply, whether specific or general, are the leaves and the apical bud, since the removal of them affects both the activities alike. The root formation is related not merely with the increased carbohydrate content at the treated region but on the high value of the ratio of the total carbohydrate and the reducing sugar.

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REFERENCES

[1] Guha-Thakurta, A. and B. K. Dutt.—Effect of high concentration auxin on the Growth and Geotropism of the coleoptile (Triticum). Trans. Bose Res. Inst., 13: 215–254. 1937-38.

[2] Cooper, W. C.—Hormones in relation to root formation on stem cuttings. Plant Physiol., 10: 789—794. 1935.

————Transport of root forming hormone in woody cuttings. Plant Physiol., 11: 779-793. 1936.

————Hormones and root formation. Bot. Gaz., 99: 599-614. 1938.

[3] Bouillenne, R. and F. W. Went.—Researches experimentales sur la neoformation des racines dans le plantules et les boutres des plantes superieures. Ann. Jard. bot. Buitenzorg., 43: 25-202. 1933.

[4] Nemec, B.—Ernahrung, Organogene und Regeneration. Vest. Kral. Ces. Spol. Nauk. Ir., 11: 1-34. 1934.

[5] Thimann, K. V. and J. B. Koepfli.—Identity of growth promoting and root forming substances of plants. Nature, 135: 101. 1935.

[6] Kogl, F.—Uber Wuchsstoffe der Auxin-und der Bios-Grouppe. Ber. d. chem. Ges., 68: 16-28. 1935.

[7] Went, F. W.—Specific factors other than auxin affecting growth root formation. Plant Physiol., 13: 55-80. 1938.

[8] Fischnich, O.—Die Rolle de Wuchsstoffes bei der Bildung von Advestivsprossen und-wurzelm. Ber. Deutsch. Bot. Ges., 56(4): 144-152. 1938.

[9] Fischnich, O.—Weiter Versuche uber die Bedeutung des Wuchsstoffes fur die Adventivspross—und Wurzelbildung. Ber. Deutsch. Bot. Ges., 57: 122-134. 1939.

[10] Stuart, Neil W.—Nitrogen and Carbohydrate metabolism of kidney bean cuttings as affected by treatment with indole acetic acid. Bot. Gaz., 100(2): 298-311. 1938.

[11] Alexander, Taylor R.—Carbohydrates of bean plants after treatment with indole acetic acid. Plant Physiol., 13(4): 845-858. 1938.

[12] Harrison, Bertrand F.—Histological responses of Iresine lindinii to indole acetic acid. Bot. Gaz., 99(2): 301–338. 1937.

[13] Hamner, Karl C.—Histological responses of Mirabilis jalapa to indole acetic acid. Bot. Gaz., 99(4): 912–954. 1938.

[14] Dorn, Hans.—Histologische Studien uber die Entwicklung sprossburtiger Wurzeln nach Hetero-auxinbehandlung. Planta., 28(1): 20–42. 1938.
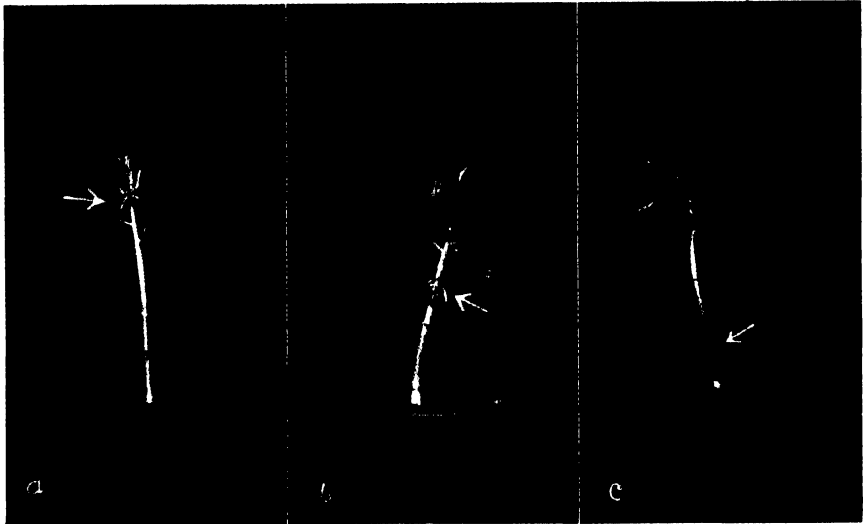
FIG. 3   Photographs showing root formation in different regions of Impatiens stem by
treatment with high concentration auxin ; (*a*) apically treated ; (*b*) centrally
treated ; (*c*) basally treated.
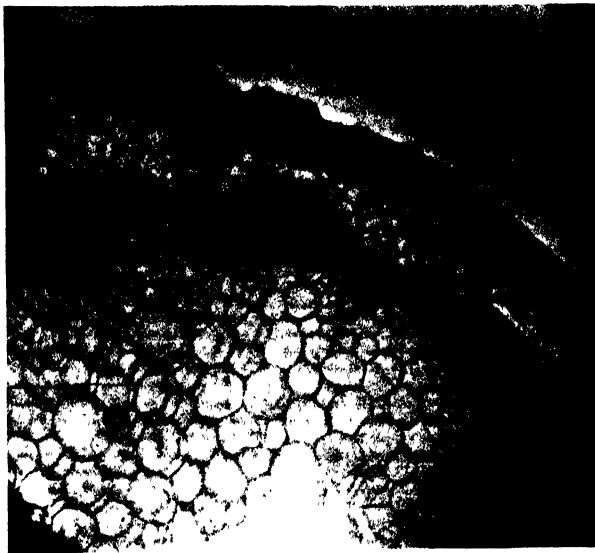
FIG. 5.   Photo-micrograph of cross-section of Impatiens stem on the third day of treatment
with high concentration auxin, showing rudiments of roots at two portions
of the interfacicular cambium.   (Magnified 40 times)
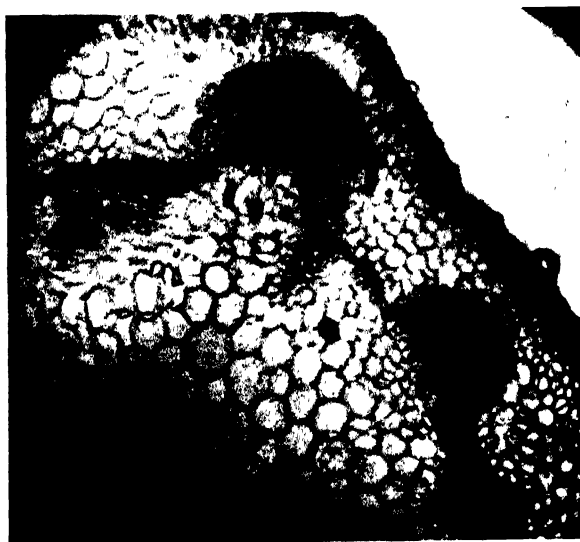
FIG 6   Photo-micrograph of cross-section of Impatiens stem on the fifth day of treatment
with high concentration auxin, showing two rudimentary roots fairly enlarged.
(Magnified 40 times.)

FIG. 7.   Photo-micrograph of cross-section of Impatiens stem on the seventh day of treatment
with high concentration auxin, showing two developed roots protruded out of
the epidermis.   (Magnified 40 times.)

FIG. 8. Photo-micrograph of cross-section of the apical portion of the stem of the control plant, showing 18 vascular bundles. (Magnified 20 times.)

FIG. 9 Photo-micrograph of the cross-section of the central portion of the stem of normal plant, showing 11 vascular bundles. (Magnified 20 times)

FIG. 10. Photo-micrograph of the cross-section of the basal portion of the stem of normal plant, showing 6 vascular bundles. (Magnified 20 times.)

VI. STUDIES IN THE PHYSIOLOGY OF SOME INDIAN FRUITS

I. THE AVAILABLE SUBSTRATES IN A DEVELOPING FRUIT OF GUAVA AND THEIR BEHAVIOUR UNDER CONDITIONS OF STORAGE AND ETHYLENE TREATMENT

By B. K. KAR, M.Sc., Ph.D. *and* H. K. BANERJEE,* M.Sc.

I. INTRODUCTION

A series of investigations was conducted to investigate the various aspects of the physiology of development and ripening of some of the Indian fruits, and the behaviour of the substrate concentrations under different environments and storage conditions. Experiments were carried out on the fruits of *Psidium guyava* and *Mangifera indica*, the two most widely spread and cultivated orchard plants in this country. In the present report the results so far obtained from experiments on guava are discussed and the other aspects of such investigations will be reported in subsequent papers.

Psidium guyava is widely spread both as cultivated and wild varieties, which are so numerous that all grades of sizes and from bitter sour to delicious sweet taste are met with. Due to its wide habitat and easy availability the fruit has secured a place of economic importance. The fruit like other tropical fruits has to pass through uncertain climatic conditions and the sequence of physio-chemical changes leading to maturity and ripening is so uncertain and rapid that the handling of the fruit in storage is confronted with serious difficulties. It was therefore thought worth while to study the response of the fruit to different environments, both in nature as well as under controlled and artificial conditions. A detailed knowledge of the substrate behaviour of the fruit in nature and under experimental conditions will give results of great importance in solving successfully the storage problem. The commercial aspect of cold storage of Mangoes has recently received some attention from Cheema and Karmarkar[9] and some metabolic problems have been worked out by Singh and collaborators[44, 45]; and by Ranjan and others[42]; but the chemical nature of the available substrates and their behaviour during metabolic processes in the fruit under different storage conditions have not yet been fully worked out.

In a study of the behaviour of a fruit to its environments it must be noted that we are concerned with a vital organism. Whether in cold or in gas storage it reacts not only to the atmosphere of the storage, but also to its own

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internal atmosphere.   The fruit in its different stages gives out certain quantities of water vapour, absorbs oxygen, gives off CO_2, certain volatile products and generates heat.   These activities depend to a great extent upon the stage and the nature of the metabolic changes in the substrates of the fruit in question. The fruits in their life-cycle show well-defined stages with their characteristic chemical substrates (Kidd and collaborators [29, 30], Gustafson [19, 20]).   The study of the respiration rate has also shown distinct respiratory stages in the life of the fruit (Blackman [5]).   The respiratory activity may be taken as an index of the substrate concentration which is correlated with the stage of the fruit.   So a study of the time of gathering, the chemical history and the nature of the delicately balanced fruit substrate have made useful contributions towards the successful handling of the storage problem.   Work has been done on the chemical changes in such fruits as oranges, cherries, grapes, peaches, pears, figs, dates, melons, and especially detailed work has been done on apples by Archbold [1, 2, 3, 4] and others.   The acid metabolism in apples has been worked out by Haynes [28].   Side by side with the chemical changes in the substrate concentration, the atmosphere produced by the evolution of gaseous substances are of considerable importance to the fruit in storage. The study of the gaseous interchange has indicated a successful method of gas storage and the discovery of the fact that volatile products such as ethylene evolved by the fruit has been for a long time utilized as a means of artificial ripening by fruit growers.

Denny [11] found that the colouring in lemons was very effectively and quickly produced by use of very low concentrations of ethylene.   Since then the evolution of ethylene gas with other volatile products of the fruit has been demonstrated by Denny [11], Denny, Miller and Lawrence [12] as indicated by the epinastic response of leaves.   Elmer [15] showed the inhibition of growth in potato caused by the gas emanating from apples.   Gane [21] reported the presence of ethylene by its effect in stunting growth of pea seedlings.   Kidd and West [30], (1935) have secured evidence of the production of ethylene in unripe immature apples.   The evolution of ethylene from fruits and its effectiveness in enhancing fruit ripening indicates that ethylene, which is itself a bye-product of internal metabolic changes, when applied externally causes far-reaching physiological changes in the fruit substrates.

The metabolic changes caused by artificial doses of ethylene have been investigated by several workers, but the results reported so far are not uniform and the real nature of ethylene reaction and its relation to metabolites in inducing fruit ripening are not clearly understood.   The effect of ethylene in artificial ripening has been studied by Denny [11] on lemons; Harvey [24, 25] on celery and bananas; Wolfe [46] and Hartshorn [26] on bananas; Davis and Church [10] on Japanese persimmons; Elmer Hansen [15] on pears; Kidd and West [30] (1933) on apples; Work [47] and Rosa [41] on Tomatoes; and Regeimbal and Harvey [48] on pineapples.   The oxidative nature of ethylene reaction as shown by an increase in respiration rate (Denny [11] and Hansen [15]); by increased rate of

starch hydrolysis (Harvey [24, 25] and Hansen [16]); by increase in sugar content and the rapid transformation of proto-pectin to pectin (Hansen [15] and Emmett [17]) have been reported.   Ethylene-treated pineapples were sweeter and there was greater activity of the proteolytic enzymes than the untreated ones (Regeimbal and Harvey [43]).   Hartshorn [36] found increased rates of respiration, starch hydrolysis, flavour and colour changes in treated bananas.   Other investigators as Chace and Church [8], Hibbard [27], Wolfe [46] have observed no increase in carbohydrate content by ethylene treatment.   Harvey [24, 25] has suggested that these differences in reaction might have been due to the very low concentration of ethylene gas used.   Dustman [14] found that stored apples were not affected chemically when treated with ethylene and ethylene-chlorohydrin but it hastened colour changes   Work [47], Hansen and Hartman [28] and Hansen [16] have found that the response obtained by ethylene varied greatly according to the respiratory activity and the stage of maturity of the fruit at the time of treatment.

From the results of the investigations enumerated above, it appears to have been realized by some of the later workers that the effect of ethylene is dependent upon more than one factor and the conflicting results obtained are primarily due to the difference in the nature of the experimental material. As pointed out before, that any study of the fruit-reaction to its environment, the vital nature of the fruit and its delicately balanced chemical substrates should not be lost sight of.   The fruit-physiology is dependent upon more than one factor and the different aspects should be considered as links in the chain of a series of reaction.   In order to have a complete picture of the problem the experiments were conducted to throw light on the following aspects: (1) the changes in the available substrates during the different stages in the growth of the fruit in nature, (2) the behaviour under storage of the substrates from the fruits collected at different pickings, (3) the effect of ethylene on the chemical substrates as a means of artificial ripening.   The results obtained have been described in a way as to make prominent the importance of the different aspects and at the same time their interdependence with each other so that the complicated metabolic changes taking place in the fruit may be properly understood.

II. MATERIALS AND METHODS

Collection and Sampling : The fruits of *Psidium guyava* were collected after intervals of two weeks beginning from the earliest to the fully ripened stage.   In order to get uniform material the fruits were collected as far as possible from a single tree throughout the period of experiment.   In between the main pickings fruits were also collected from different trees in order to carry out isolated experiments to verify the main results.   Great care was taken to collect, as far as possible, in each picking fruits of the same age and size.   This was made possible to some extent by earmarking the fruits from the earliest stages.   100 fruits were collected in each picking and seven pickings were made which when viewed together roughly corresponded to the

different well-marked stages of the fruit. These stages were further verified on the basis of the chemical constituents of the substrates during the progress of the investigations. All the pickings were made in the morning and kept in the laboratory for 5-6 hours under an uniform temperature of about 28°C. The fresh weight of the fruits were taken to obtain an average weight of the samples per picking.

Next by random selection they were sorted out in four groups according to the requirements of the experiments, viz. group 1 for fresh analysis; group 2 for storage under room temperature of 28°-32°C.; group 3 for cold storage in a refrigerator set at a temperature ranging from 8°-12°C., and group 4 for ethylene treatment. Ethylene gas was prepared in the laboratory from ethyl alcohol and H_2SO_4 (conc.) and collected over water in a gas container of 20 litres capacity after being washed through bubbling in conc. KOH and pyrogallol solution. Required volume of this ethylene gas necessary to make a concentration of 1 : 1000 by volume was passed into an inverted big wide glass jar containing the fruits. In another glass jar the fruits were stored without ethylene as the control set. Both were kept under room temperature.

To prevent accumulation of CO_2 inside the jars a petri dish containing concentrated KOH solution was kept inside each jar. In addition to this the ethylene treated fruits and the control set were allowed complete aeration by keeping the jars open to the atmosphere for 2 hours every day. Then the required quantity of ethylene was again introduced inside the jar to keep the original concentration. This process of aeration and ethylene treatment was repeated for 4 days and then the treated fruits were allowed to remain in storage without ethylene atmosphere and as in untreated control ones. The concentration of ethylene and its period of treatment was adjusted after preliminary experiments, so that there may not be any possibility of scald formation. The above-described storage conditions and ethylene treatments were continued from 8-10 days as it was found that the fruits were liable to be injured if the period of treatments were prolonged. The samples were drawn on alternate days from each treatments and the chemical analysis of the constituents were made. The samples from all the pickings were treated similarly as described above.

Methods of analysis: The fruits were divisible into two portions: (i) outer pulp, (ii) inner core containing seeds. Analysis was made separately with the outer pulp. The outer pulp was cut into small pieces and then crushed together in a mortar. 25 gm. from each were extracted with about 200 c.c. 80% alcohol in a soxhlet apparatus for about 24-30 hrs. The alcohol was then evaporated under reduced pressure over a water bath at a temperature below 50°C. The residue was taken in warm distilled water and made up to 100 c.c. in a graduated flask. Free acid and sugars were estimated from this extracted solution, and from the alcohol insoluble residue remaining in the thimble, starch was estimated.

Free acid was determined on 20 c.c. of the extract by titration with N/10 NaOH. Phenolphthalein was used as an indicator.

Reducing sugars : Definite volume from the extracted solution was almost neutralized with N/10 NaOH and cleared with basic lead acetate and saturated Na-phosphate solutions (Archbold and Widdowson [48]). The solution was filtered and made up to volume. Reducing sugars were determined in aliquots of this cleared filtrate by the copper titration method of Lane and Eynon [34].

Sucrose : A definite volume of the cleared filtrate was inverted with 10% HCl according to Martin [36, 37] neutralized with Na_2CO_3 and the invert sugar estimated by the above copper titration method. The sucrose value was obtained by the difference in reducing sugar before and after inversion.

Alcohol insoluble residue : This was determined by drying in a steam chamber (60°C.) the material remaining in the thimbles after extraction until constant weight.

Starch was determined on 1 gm. of the alcohol insoluble residue finely ground in a mortar and digested over night with fresh saliva according to the method described by Loomis and Shull [35] and also adopted by Hansen [15]. The digested material was taken into solution in water, cleared by basic lead acetate and deleaded by Na-phosphate. The filtrate was hydrolyzed by HCl and neutralized with Na_2CO_3. The reducing values were determined by the copper titration method referred to above. Starch was calculated from this value by multiplying by the factor 0·90.

Nitrogen estimation : Nitrogen in 1 gm. of fresh fruit material was estimated according to the Kjeldahl technique and the protein calculated from the nitrogen value by multiplication with the factor 6·4.

III. EXPERIMENTAL RESULTS

The results obtained have been described under three sections according to the nature of the investigations. The first section deals with the natural drift of the substrate concentration when the fruit was growing in tree under natural conditions. The second section deals with the response of the substrates from different pickings under storage conditions and the third section deals with the changes in the substrate concentration under an artificial atmosphere of ethylene. The results have been considered together in the discussion.

Section 1 : The natural drift of the substrate concentration in the ontogeny of the fruit.

The fruits were collected from Dum Dum Nursery during the months of July, August and September. Fruits collected in July were of very early stage when two pickings were made after an interval of ten days. In August the fruits were growing with rapid increase in size, the intercellular spaces appeared and the fruit became glossy and light green in colour. Three pickings were made in August. In September the fruits were fully grown, attaining the maximum size, it was possible to have two pickings during this month.

In Table I the different picking dates with corresponding fresh weights of the fruit and their moisture contents have been arranged.

TABLE I

Picking dates		Fresh weights (average of 25 fruits)	Percentage moisture content on fresh weight of pulp
July 10, 1939	.	18 gm.	40
July 20 ,, ..	..	24 ,,	45
Aug. 2 ,, ..	.	50 ,,	68
Aug. 12 ,, ..	.	60 ,,	71
Aug. 25 ., ..	.	80 ,,	79
Sept. 5 ,, ..	..	97 ,,	84
Sept. 10 ,, ..	..	98 ,,	84

Showing average fresh weight and percentage moisture content in different pickings.

The growth of the fruit within a period of 62 days showed an increase of six times in fresh weight and a corresponding doubling in the moisture content. This was the case when only the outer pulpy portion of the fruit was considered. The chemical analysis of the pulp from fruits collected at different pickings have been given in Table II.

TABLE II

Different pickings from the same tree

Different substrates	July		August			September	
1. Protein .	0·65	0·63	0·50	0·43	0·37	0·35	0·34
2. Starch	0·06	0·08	0·15	0·16	0·10	0·04	0·02
3. Total sugar	0·41	0·43	2·76	3·00	3·00	3·50	3·25
4. Reducing sugar	0·35	0 36	1·85	2 10	2·00	2·40	1·50
5. Sucrose	0·06	0 07	0·91	0·90	1·00	1·10	1·75
6. Titrable acid (as citric acid) ..	0·07	0·09	0·30	0·32	0·30	0·26	0·26
7 Alcohol insoluble residue ..	26·87	26·60	22·00	20·00	17·46	15·00	11·60

Substrate concentration in the different stages of the fruit during its life-cycle in tree. Calculated on the basis of percentage of fresh weight of the pulp.

The drift of the chemical substrates as represented in the Table II showed that in the samples of the July pickings a higher protein content was found. The starch was found only in traces while total and reducing sugars were present next in amount to protein. The sucrose value was very low. Traces
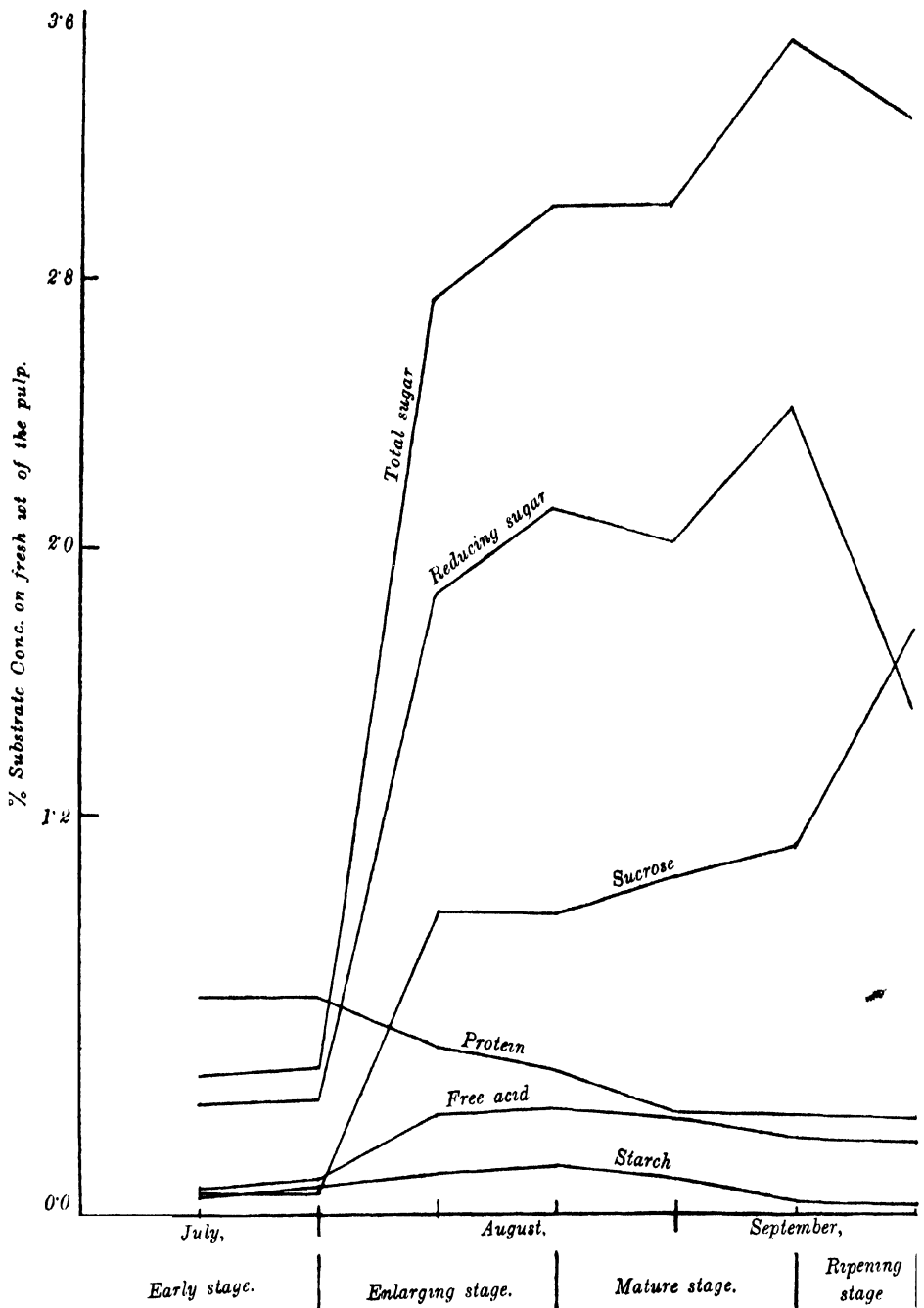
Fig. 1. — Showing the metabolic drift of the various substrates during the life-cycle of the fruit in the tree.

of free acid was present and about 27% alcohol insoluble residue was noted. So amongst the chemical constituents estimated the amount of protein was found to be the chief constituent associated with the fruit in its early stage with 40–45% moisture content and the substrate found in appreciable amount next to protein was total sugar, of which the sucrose content was very low. With these amounts of substrates at its disposal the fruit enters the stage of rapid growth in size with appearance of intercellular spaces.

Associated with this stage of the fruit it was found that in August pickings a marked difference in substrate concentration was noted as compared to the early stage. The protein content showed a decrease by 1/3 while the other substrates increased considerably. The starch which was present only in traces in the early stage showed a steep rise and other carbohydrates such as total sugar also increased by about 6 to 7 times the previous value. A marked increase in sucrose content was also noticed. The acid content increased from 0·07% to 0·30% but the alcohol insoluble residue decreased by 5%. The marked increase in carbohydrate contents was very significant which indicated the association of sugars as respirable substrates for the increased metabolic activities of the growing fruit in this stage of its life-cycle. The loss in protein content may be explained to some extent by the increase in carbohydrates.

The increase in the available chemical substrates was maintained in the samples of the subsequent pickings of the mature stage. In this stage the higher values of total and reducing sugars were maintained but protein value was further lowered. Starch which had a sharp rise in the previous stage declined again to a low value. Sucrose content further increased while acid and alcohol insoluble residue showed a decrease. Therefore the enlarging and mature stages of the fruit were characterized by higher percentage of glucose and sucrose with less protein content. Starch which increased sharply in one stage of the fruit had also a sharp fall in the later stages. There was a regular decrease in the value of the alcohol insoluble residue.

The drift in the available substrates in the last picking in September where the fruits were fully mature and in the ripening stage was in the down grade direction. Protein further decreased to a lower value and amongst the carbohydrates, only traces of starch was left and reducing sugar also showed a decrease while sucrose content kept more or less the same level. The free acid content remained constant and alcohol insoluble residue showed further decrease to a minimum value. In Figure 1, the metabolic drift in the life-cycle of the fruit has been graphically represented.

Considering the change in the individual substrates accompanying the change over from one stage to the other, it was found that the first change over from early stage to the enlarging stage was a very marked one compared to the rest. In the Tables III and IV the average substrate concentrations in the four main stages of the fruit and their change from one stage to the other have been given respectively.

TABLE III

Different substrates	Early stage	Enlarging stage	Mature stage	Ripening stage
1. Protein	·64	·47	·36	0·33
2. Starch	·07	·15	·07	0·02
3. Total sugar	·42	2·88	3·25	3·25
4. Reducing sugar	·35	1·96	2·20	1·50
5. Sucrose	·07	0·91	1·10	1·75
6. Titrable acid (as citric acid)	·08	0·32	·28	·26
7. Alcohol insoluble residue	26·74	21·50	16·24	11·60

Showing average percentage substrate concentrations in different stages.

TABLE IV

	Protein	Starch	Total sugar	Reduc- ing sugar	Sucrose	Titrable acid	Alcohol insolu- ble residue
1. Present in July (early stage)	·64	·07	·42	·35	·07	·08	26·74
2. Change between July and August (early to enlarging stage)	-·17	·09	2·47	1·61	·84	·24	-5·24
3. Change between (Aug. and Sept. enlarging to mature stage)	-·10	-·09	1·40	0·24	0·19	-·10	-5·26
4. Change from mature stage to ripening stage	-·03	-·05	0·00	-0·70	0·65	-·02	-4·64

Showing percentage substrate concentrations during 'change overs' from one stage to the other.

It will be seen that the change in the individual substrate during the first change over from early to the enlarging stage is positive in all cases except in that of protein and alcohol insoluble residue. The maximum increase was noted in total sugar. In the second change from enlarging to mature stage only the total sugar including glucose and sucrose showed increase while protein and starch decreased. So starch and acid which were positive in the first change over now showed negative change. In the final change-over from mature to ripening stage all the substrates showed negative change excepting sucrose. Therefore considering the complete life-cycle of the fruit, the various chemical substrates behaved differently at different stages of the fruit. The protein-drift showed a gradual decrease throughout, the decrease was more at the time of earlier changes to rapid growth and development than in the later stages. Corresponding with the change in protein, the alcohol insoluble residue decreased with nearly equal amounts of change throughout. It was with the carbohydrates that fluctuating drifts were noted and maximum change obtained during the change over to active growth and development.

In the ripening stage a declining drift in all the substrates was the feature. In the next section, the results obtained from experiments on the fruits collected at different stages under cold storage have been discussed.

Section 2 : The drift of the substrate concentration in the different stages of the fruit in cold storage.

Fruits collected at different pickings were divided into three sets one kept in cold storage in a refrigerator at a temperature ranging from 8°–12°C. and another set was placed under room conditions of 28°–32°C. which was considered as control for the cold storage set. The third set was treated with ethylene, which is described in the next section. The fruits were kept in storage for 7 days and the samples were drawn from it and the different substrates were estimated as before. In Table V the behaviour of the various

TABLE V

Original substrate concentration 100

Various substrates	Concentration at the end of a period of 7 days			
	Early stage	Enlarging stage	Mature stage	Ripening stage
1. Protein	C—42·85	83·74	84·51	60·23
	R—39·25	73·00	72·92	52·47
2. Starch	C—19·02	51·39	41·49	28·73
	R—14·80	43·81	32 67	23·42
3. Total sugar	C 62·36	92·09	180·74	165·52
	R—53·37	76·17	172·47	163·46
4. Reducing sugar	C—52·78	88·00	151 48	129 75
	R—39·30	66·67	149·32	130·13
5. Sucrose	C— 9·58	4 09	29·26	35·77
	R—14·07	9·50	23·15	33·33
6. Titrable acid (as citric acid)	C—86·60	91·40	86·00	82·45
	R—78·12	82·30	82·00	81·12
7. Alcohol insoluble residue	C—28·00	18·40	20·48	12 00
	R—24·00	18·88	12·20	11·00

Showing substrate concentration in different storage condition –
C—Cold storage, temperature 8°–12°C.
R—Stored in a room, temperature 28°–32°C.

substrates during cold storage as compared to those kept under room conditions for the different pickings are summarized. In Table VI the difference in substrate concentration due to cold storage has been given for the different stages of the fruit. It is necessary to describe at this stage how the datas tabulated were obtained. For comparison the values represented in the Tables were derived in the following way : Firstly, the average substrate concentration of the samples gathered at different pickings were obtained before

submitting them to treatments. (These values were regarded as original substrate concentration). Secondly, the average concentration of the various substrates due to storage after the required period were obtained. Lastly, the change due to storage or to ethylene treatment were calculated by taking the original substrate concentration as 100 for each individual substrate estimated.

Considering the results tabulated in Tables V and VI, the general effect of the cold storage in fruits gathered at different pickings was seen to be the higher concentration of the individual substrates in the fruits kept in cold storage than in the corresponding storage under room conditions.

TABLE VI

Various substrates	Concentration at the end of a period of 7 days			
	Early stage	Enlarging stage	Mature stage	Ripening stage
1. Protein	3·60	10·74	11·59	7·76
2. Starch	4·22	7·58	8·82	5·31
3. Total sugar	8·99	15·92	8·27	2·06
4. Reducing sugar	13·48	21·33	2 16	0·00
5. Sucrose	−4·49	−5 41	6·09	2·44
6. Titrable acid (as citric acid)	8·48	9·10	4·00	1 33
7. Alcohol insoluble residue	4·00	0·00	8·28	1·00

Showing change in substrate concentration due to cold storage (cold storage—room storage) in fruits collected at different stages.

Fruits under storage conditions may be taken as in a state of starvation and it may be expected that after a certain period of storage, the metabolic drift in the stored fruits with respect to certain metabolites, would show a declining tendency depending upon the state of the fruit and the intensity of its respiration. There would be a constant drain on the available respirable substrates. This was found by the steady loss in substrate concentration, both in cold as well as in room storage from the original substrate concentration. But the higher percentage concentration found in cold storage showed that the drain was arrested and slowed down under cold storage than under room conditions. The drift under cold storage was flattened and prolonged over a wider period. Within this general behaviour, the individual substrate behaved differentially, depending upon the stage of the fruit under storage.

In the fruits of the early stage, all-round minimum values were obtained in case of almost all the substrates both under cold as well as in higher temperature storage, than in the subsequent stages of the fruit. This may be attributed to the immature stage of the fruit at this stage. As noted before in Section 1 the fruits at this stage consisted mainly of protein and small quantities of carbohydrates. This nature of the chemical substrates of the fruit was probably not suited to storage as the highly elaborated chemical

substrates found in the later stages of the fruit. Taking the individual substrate into consideration it was found that protein substrate reacted almost similarly in both kinds of storage and there was not much difference (3·60) at the ultimate end of the storage. But the reaction of the carbohydrate substrates were different and great fluctuations were noted. Starch was hydrolyzed more in higher temperature storage than in cold and the corresponding sugar contents showed wide variations. Reducing sugar behaved like starch having greater percentage concentration in cold storage while sucrose content showed lower concentration. The same behaviour was also found in the next enlarging stage where the reducing sugar was much more diminished in room condition than under the same condition in the early stage, while sucrose showed the same increase under room condition. This may be due to the amounts of starch present, the available sugars produced due to hydrolysis of starch and the drain on them according to the stage of the fruit. This may also be due to traces of starch and sucrose present initially before storage in this early stage. The titrable acids reacted similarly in these two stages of the fruit.

In fruits of the mature stage the reactions of carbohydrates to different storage conditions were much narrowed down, and the great variations noted in the preceding stages were absent. Sucrose behaved similarly as the other sugars. Protein content diminished to the same extent as in previous stage. In all the stages the titrable acid varied similarly within a certain range but on the whole the rate of decrease was lessened in cold storage. The variations in the alcohol insoluble residue throughout showed no well-defined tendency of reaction. The fruits in the ripening stage showed no marked difference in reaction in their chemical substrates due to storage conditions. Fruits from subsequent developmental stages when placed under same conditions of storage, the chemical substrates in the fruit react differently according to the developmental phase of the fruit. Within a period of 7 days of storage it was found that variations were more in the earlier stages of the fruit than in the later stages. The variations were more marked in protein and carbohydrate substrates than in other substrates, and sucrose specially showed a minimum value under cold storage in the early stages, but reacted like glucose in the later stages.

Section 3 : Effect of artificial doses of ethylene on the substrate concentration in the fruits collected at different stages.

Before considering the effect of ethylene gas on the substrate concentration it was thought worth while to determine whether ethylene was at all emanated from the fruits in course of natural ripening. This was done by placing germinated pea seedlings in an atmosphere created by ripening guavas. It was found that pea seedlings had stunted growth as was found by previous workers, (Elmer, Gane). The emanation of ethylene from ripening guavas manifested itself and also could be detected by the strong sweet odour similar to ethylene, produced at the time of ripening. The factor of ethylene emanation in nature, during the process of ripening as a result of metabolic changes,

should be considered as a very important factor in all such investigations where artificial effect of ethylene was to be studied.   The detailed investigations on this aspect of the problem of ethylene emanation and its relation with the substrate concentration will be discussed later in a separate communication. At present the effect of artificial doses of ethylene on the different stages of the fruit and the reaction of the available chemical substrates has been studied. In Table VII the substrate concentration as a result of 7 days of ethylene treatment (method of treatment has been described before) on the fruits collected at different pickings are summarized.

TABLE VII

Original substrate concentration 100

Various substrates.	Substrate concentration at the end of a period of 7 days			
	Early stage	Enlarging stage	Mature stage	Ripening stage
1. Protein	R—39·25 E—21·43	73·00 62·53	72·92 51·26	52·47 48·76
2. Starch	R—14·80 E—10·75	43·81 40·45	32·67 16·30	23·42 8·24
3. Total sugar	R—53·37 E—38·85	76·17 76·17	172·47 439 17	163·46 336·00
4. Reducing sugar ..	R—39·30 E—31·41	66·67 73·30	149·32 330·20	130·13 234·30
5. Sucrose	R—14·07 E— 7·44	9·50 2·87	23·15 108·97	33·33 101·70
6. Titrable acid (as citric acid)	R—78·12 E—49·74	82·30 80·00	82·00 65·12	81·12 66·41
7. Alcohol insoluble residue	R—24·00 E—23·00	18·88 16·88	12 20 13·60	11·00 9·40

Showing substrate concentration under ethylene treatment in fruits collected at different stages—

R —Stored in a room, temperature 28°C.–32°C.

E—In an atmosphere containing 0·1 % ethylene; temperature 28°C.–32°C

The effect of ethylene on the fruit, as indicated by the behaviour of the various chemical substrates, was found to vary in different stages of the fruit. The results were not uniform.   The various substrates behaved differently in different stages.   In the immature fruits of the July pickings, the substrate concentration in all cases were diminished as a result of ethylene treatment when compared with the corresponding set of untreated fruits.   The fruits which were compact and hard at this stage were in some cases scalded under the same conditions of treatment.   Some of the scalds were of superficial nature and some penetrated into the fleshy portion.   The effect of ethylene

gas as was shown by the outward effect and the general appearance of the fruits of different pickings during 7 days of treatment are given in Table VIII.

TABLE VIII

| Stages of the fruit | Percentage of fruits outwardly affected at the end of a period of 7 days in | | |
	Ethylene treated	Non-treated	REMARKS
1. Early	0–5%	Nil	Fruits were scalded and some showed yellow colouring, but were tasteless and odourless. Scalded fruits were harder than yellow turned fruits.
2. Enlarging	10–15%	Nil	No scalding, affected fruits were yellow in colour, soft, and sweet in odour and taste.
3. Maturity	25–30%	0–5%	Affected fruits were bright yellow in colour, with strong sweet taste and fragrance than the ripened fruits in non-treated ones.

Showing outward and general appearance of the fruits in ethylene treatment.

Judged from the outward effect and appearance, it seemed that the ethylene treatment, as regards the hastening of the ripening process, was more positive in mature stage of the fruit than in earlier stages. Some fruits of the early stage showed yellow colouring but the corresponding taste was not like the one ripened naturally or in ethylene treatment from later stages of the fruit. Yellowing of the fruit could therefore be primarily due to ethylene treatment; but the sweet taste like the ripened fruits could be only produced by the reaction of the substrates to the ethylene treatment, and this was more conveniently done in the later stages of the fruit. The scald formation in the early stages might be due to more than one reason. The physical nature (hard and compact) of the fruit at this stage may be one of the reasons, because the ethylene concentration (0·1%) used was common throughout the investigation. The ethylene reaction primarily sets in by diffusion of the gas into the cells of the fruit and the hard and compact nature of the fruit without any inter-cellular spaces in the early stage perhaps makes this diffusion process difficult.

In these investigations two experimental conditions were to be considered for the analysis of the substrate behaviour. Firstly, the changes brought about by the storage of the fruit and secondly, the changes due to the ethylene treatment. In ethylene treated set both the conditions were responsible for the changes brought about in the chemical substrates. In Table IX the changes in substrate concentration due to ethylene treatment have been given:

TABLE IX

Various substrates.	Substrate conc. at the end of a period of 7 days			
	Early stage	Enlarging stage	Mature stage	Ripening stage
1. Protein	−17·82	−10·47	−21·66	− 3·71
2. Starch	− 4·05	− 3·36	− 6·37	−25·18
3. Total sugar	−14·52	0·00	266·70	172·4
4. Reducing sugar	− 7·89	6·63	180·88	104·17
5. Sucrose	− 6·63	− 6·63	85·82	68·37
6. Titrable acid (citric acid) ..	−28·38	− 2·30	−16·88	−14·71
7. Alcohol insoluble residue ..	− 1·00	− 2·00	1·40	− 1·60

Showing changes in substrate concentration due to ethylene treatment as compared to the untreated ones.

(Conc. in untreated—conc. in ethylene treated.)

The results represented in Table IX showed the irregular behaviour of the substrate concentration in different pickings. Protein substrate showed a decrease in all the stages of the fruit as a result of ethylene treatment. The greatest decrease was noted in the mature stage. The same was the reaction with starch, i.e. decreased all along in ethylene treated set. The effect of ethylene was only shown in the mature and ripening stages of the fruit as indicated by rise in different sugar contents. The titrable acid and alcohol insoluble residue also decreased. Therefore considering the whole life-cycle of the fruit as represented by the different stages of the fruit collected in these investigations, the effect of ethylene was noticeable only in the later stages of the fruit and the conflicting results obtained by the previous workers was perhaps due to the nature of the experimental fruit. When different stages were considered here separately, the difference in behaviour of the individual substrates became more prominent. In early stage of the fruit all the substrates showed a more or less rapid decrease and the effect of ethylene could only be seen by the rapid decline of the substrate concentration and not by any increase. This was quite contrary to the results obtained in the later stages of the fruit. As noted before, the all-round declining of the substrates in the early stage was perhaps due to the scald formation in some cases and due to the nature of the fruit at this stage. Therefore the definite sequence of changes at this stage of the fruit could not be detected in this investigation. But in the next enlarging stage an appreciable increase in reducing sugar was obtained, which showed a greater increase in the mature stage. In this stage a strong hydrolysis of starch was accompanied by a maximum increase in different sugars. The greatest drain was made on the protein content. The increase in sugars was maintained in the ripening stage. Therefore leaving the earliest stage where no definite sequence could be obtained, all the subsequent stages of the fruit were amenable to some sort of ethylene reaction as indicated in these investigations by the strong depletion of protein, by strong hydrolysis of starch and
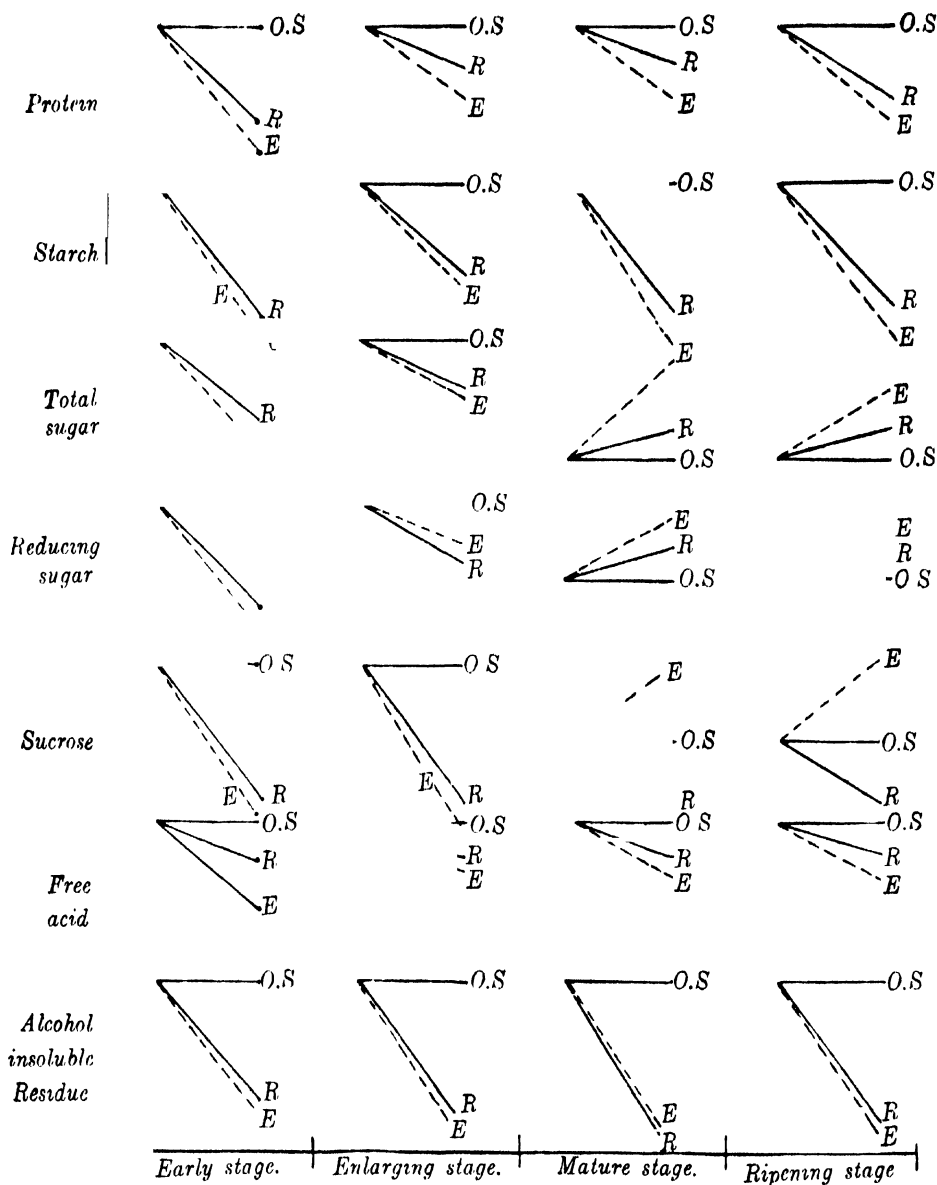
FIG. 2.—Showing comparative changes in the various substrate concentrations due to ethylene action. O.S—original substrate ; R—under room conditions, 28°C.-32°C. ; E—under ethylene treatment and temperature 28°C.-32°C. The rise and fall in substrate concentration is indicated respectively above or below the O.S line.

corresponding increase in total and reducing sugars. But the ethylene reaction was found to be more prominent on the chemical substrates provided by the fruit in the mature stage. The respiratory activity of the fruit which may be in the climacteric region in this mature stage was perhaps also responsible for the metabolic activities shown in these investigations. The comparative change in the substrate concentration due to ethylene action has been represented graphically in figure 2.

It is known that climacteric rise is accompanied by certain evolution of volatile products and this may have an effect on the change in substrate concentration as noted in the mature stage, and the artificial doses of ethylene was only responsible in augmenting the change further. This aspect of the investigation is a problem for further investigation.

Discussion : From the report of the investigations it will be seen how the results obtained depended on the nature of the material collected at different pickings. The nature of guava fruits was difficult to ascertain, because in the same picking the physiological nature and the biochemical changes, leading to development and ultimate ripening, were not uniform in all the fruits of a particular picking. It was therefore not possible to expect similar and uniform results throughout, under the different experimental conditions. But the average of the results within a certain range was taken and any wide variation in the results is to be attributed to the nature of some of the fruits in the lot. But the general drift of the various chemical substrates was, however, clearly seen.

The natural drift of the metabolic changes in the guava fruit from petal-fall to the ripening stage showed well marked stages of growth and development. These stages were not only morphological in nature but showed distinct physiological and biochemical characteristics (Singh and others [44, 45]; Kidd [20]). During these investigations it was found that the passing over of one phase to the other was accompanied by distinct and far-reaching changes in the biochemical nature of the substrates. The fruit started with protein as its chief constituent, and in the first change over which was also the beginning of very active growth and development, an accumulation of soluble carbohydrates took place. Amongst the soluble carbohydrates, glucose showed a maximum increase in the maturity period after which it gradually decreased. But the sucrose content which was present only in traces in the early stage was found to increase gradually all along. The starch which was present in traces in the early stage increased rapidly in the enlarging stage and then again decreased to a minimum value. Therefore glucose and sucrose both increased with the rise in starch content in the enlarging stage, but with the hydrolysis of starch in the mature stage glucose naturally continued to rise and reached the maximum concentration but here significantly sucrose also showed an increase. In the next stage the fast disappearance of starch was accompanied by a decline in the glucose content while sucrose maintained its high level and with disappearance of starch, sucrose did not disappear. A similar behaviour of the

carbohydrates was also noted by Kidd [29] in his study on the respiration of apples. In the ripening stage the total disappearance of starch was accompanied by a reduction in glucose content and sucrose continued steadily to increase. There was a good reduction in the acid content in the later stages of the fruit. The accumulation of soluble carbohydrates in the active growth phase of the fruit, provided the demand of the available respirable substrates in the enlarging cells and this may have been derived at the cost of protein content and the consequent decrease in the alcohol insoluble residue. The protein content showed a gradual decrease throughout from the early stage to the ripening stage.

The stages differentiated in these investigations were of a great physiological importance for any study of the fruit metabolism. The highest metabolic activity in terms of starch hydrolysis and corresponding accumulation of maximum concentrations of various sugars was found in the region of highest cell-division and growth activity. The change over from early to active growth stage was very marked while the change over from mature to ripening stage was a gradual one. Naturally it was expected that fruits in their well-defined stages would react differently to the same conditions of storage.

The storage drift of the various substrates or the reaction due to ethylene treatment should therefore be considered in the light of the dynamic nature of the chemical substrates as discussed above. The biochemical identity of the fruit at the time of the experiment was a very essential factor and this has been clearly identified during the course of storage reactions and ethylene treatment. From these investigations it was seen that guava of early stages were not suitable for cold storage, because the behaviour of the chemical substrates, indicated a more rapid decrease than in the later stages of the fruit, when kept under the same conditions of storage. From the investigations on apple (Archbold [4]) a striking difference in storage behaviour was also obtained from immature and normally mature fruits. After a period of 7 days storage it was found that excepting the free acid, all other substrates in the early stage decreased to a great extent in both cold as well as room temperature storage, but the reduction was more under the room conditions. The greatest reduction was seen in starch content, and sucrose also showed a reduction in cold storage.

The sucrose drift throughout the different stages showed different behaviour in the early stages than in the later stages. Firstly, it decreased more in cold storage than under room conditions and then there was a gradual rise in cold storage, while glucose in all stages showed a higher concentration in cold storage. If the holding capacity of the fruit in storage was to be judged by the concentration of the chemical substrates left at the disposal of the fruit after a certain period of storage, then from these investigations it may be inferred that on an average the substrates showed higher substrate concentration (except in the early stages) in cold storage than under room conditions. Further the keeping of higher substrate concentration was favoured more in

the fruits verging on maturity. The higher concentration of protein in cold storage may also be due to the increase of protein nitrogen during storage as was reported by Kidd, West, Griffiths and Potter [31] in Conference Pears. The alcohol insoluble residue gave no definite indication as to its behaviour with other substrates in relation to the different physiological stages.

The results obtained for ethylene treatment, when analyzed, clearly indicated the difference in behaviour of the various substrates under the same conditions of treatment. The difference in the response of the fruits to ethylene treatment as observed by previous workers, Denny [11], Harvey [24, 25], Hansen [15], Hansen Elmer and Hartman Henry [23], Emmett [17], Wolfe [46], Regeimbal, Vacha and Harvey [43] as noted previously was perhaps due to the chemical nature of the fruits found in different pickings. In these investigations, the fruit reacted differentially to ethylene treatment while passing through the successive physiological stages. The immature fruits collected in July having protein as the chief constituent reacted in quite a different manner than the fruits collected at the end of August or the beginning of September, under the same experimental conditions of ethylene treatment. The fruits were chemically different could be seen even by the physical effect produced by the ethylene treatment. The fruits in the immature stage were to some extent scalded and those which showed yellowing were not sweet in taste as the normally ripened fruits. The ethylene perhaps acted superficially on the skin of the fruit, influencing the yellow colouration but the chemical substrates were not so to say 'chemically mature' to react positively with ethylene so as to stimulate the formation of sugars as was the case in the later stages of the fruit.

With respect to the behaviour of the individual chemical substrates, no indication of any rise in the substrate concentration in the early stage was noticed. As the fruit grows and develops with the appearance of starch and the accumulation of soluble carbohydrates, the reactivity of the fruit to ethylene treatment increased so far as the influence on the carbohydrates was considered. The first reaction to glucose content was noticed in the enlarging stage which was further continued in the next mature stage where a strong hydrolysis of starch and a corresponding increase in glucose as well as in sucrose content was found. Similar changes occurred in the ripening stage also but not to such a degree. Therefore, mature stage of the fruit was found to be the region of maximum ethylene effect. From these observations it, therefore, became clear that ethylene effect was correlated with the physiological stages of the fruit and consequently with the nature of the chemical substrates. This explains the difference in results of ethylene action found by previous workers.

The association of the maximum ethylene effect with the mature stage of the fruit raises another factor for consideration, viz. the evolution of volatile products by the fruit. The main production of the volatile product in apples coincided with the rise in respiration at the climacteric (Kidd and West [30]) or after the onset of the climacteric (Nelson [39, 40]). It was possible that the mature stage, showing maximum ethylene effect in these investigations also

coincided with the climacteric and with the main production of the volatile products in guavas. The changes in substrate concentration observed, therefore, may be initially due to the climacteric and to the main production of ethylene and the artificial doses of ethylene in that case would then only be stimulative (Nelson [38, 39]). The effect of ethylene was therefore considered to be intimately bound with the physiological stage of the fruit and consequently with the chemical substrates and with the respiratory efficiency.

The various aspect of the problem discussed here should therefore be regarded as links in the chain of a series of reactions and the variations observed during such series of investigations are to be related to the chemical identity of the experimental material and its consequent reactions to the experimental conditions.

Summary : The investigations on the physiology of *Psidium guyava* were conducted from the following aspects: (i) natural drift of the chemical substrates during the life-cycle of the fruit in tree ; (ii) the behaviour of the chemical substrates in fruits collected at different stages of maturity to (*a*) cold storage, and (*b*) to ethylene treatment. The following results were obtained during the course of the investigation :—

A. Substrate drifts under natural condition.

(1) The study of the natural drift showed distinct well marked stages of the fruit, which were not only morphological but also physiological and biochemical in nature as indicated by the difference in behaviour of the available chemical substrates.

(2) The protein drift showed a gradual decrease throughout with the maximum amount in the early stage. Starch appeared in the enlarging stage and after attaining a peak rise in the mature stage was reduced to a minimum in the ripening stage. With the hydrolysis of starch, the corresponding glucose amount increased but sucrose increased too. With disappearance of starch, glucose also decreased but sucrose maintained a higher level.

(3) Acid content fluctuated in the early stages, but showed continuous fall in the later stages. Alcohol insoluble residue showed a gradual fall throughout.

B. Substrate drifts in storage under low temperature and ethylene treatment.

(4) The fruits when subjected to storage under low temperature or to ethylene treatment, reacted according to the physiological stage of the fruit at the time of treatment.

(5) The effect of cold storage was to decrease the rate of loss of chemical substrates. The loss varied according to the physiological stage of the fruit. The maximum reduction in the various substrate concentration was noticed in the early stage.

(6) The variations in glucose and sucrose content were not similar. Glucose always showed a higher percentage in cold storage while sucrose showed a higher percentage in cold storage only in the later stages of the fruit.

(7) The effect of ethylene also varied in accordance to the stage of the maturity of the fruit. No definite effect was seen in the early stage. In subsequent stages change was noticed in (a) reduction in protein content, (b) strong hydrolysis of starch, (c) corresponding increase in total and reducing sugars (glucose tended to a greater increase than sucrose), (d) reduction in alcohol insoluble residue.

(8) The intensity of ethylene reaction increased with the maturity of the fruit and the maximum effect was seen in the mature stage.

(9) The correlation of the maximum ethylene activity with the maturity and the consequent climacteric of the fruit have been discussed, and the difference in results obtained by previous workers on ethylene reaction have been explained.

(10) Emphasis has been laid on the importance of the so-called 'chemical maturity' of the fruit for the proper investigation of storage and other problems regarding the fruit physiology.

LITERATURE

[1] Archbold, H. K.—Ann. Bot., XXXIX, 109, (1925).
[2] ———————— Ann. Bot., ibid., 77, (1925).
[3] ——— ———— Ibid., 97, (1925).
[4] ———————— Ibid., XLVI, 407, (1932).
[5] Blackman, V. H.—Science Progress, XXXIII, 417, (1939).
[6] Banerji, B. N., Karmarkar, D. V. and Row, G. R.—Agric. Liv. Stock India, 4, 36, (1934).
[7] Barnell, H. R.—Ann. Bot., N.S., Vol. IV, No. 13, 39, (1940).
[8] Chace, E. M. and Church, C. G.—Ind. and Eng. Chem., 19, 1135, (1927).
[9] Cheema and Karmarkar.—Imp. Council of Agr. Res. Bull., 21, (1939).
[10] Davis, W. B. and Church, C. G.—Journ. of Agr. Res., 42, 165, (1931).
[11] Denny, F. F.—Bot. Gaz., 77, 322, (1924).
[12] Denny, F. F. and Miller, Lawrence P.—Contrb. Boyce Thompson Inst., 7, 97, (1935)
[13] Dufrenoy, J.—Chem. Abs., 24, 2208, (1930).
[14] Dustman, R. B.—Plant Physiol., 9, 637, (1934).
[15] Elmer, Hansen.—Plant Physiol., 14, 1, 145, (1939).
[16] Elmer, O. H.—Jour. Agr. Res., 52, 609, (1936).
[17] Emmett, A. M.—Ann. Bot., 43, 269, (1929).
[18] Guthrie, T. D.—Contr. Boyce Thompson Inst., 4, 131, (1931).
[19] Gustafson, F. G.—Amer. Jour. Bot., 17, 1011, (1930).
[20] ——— - ————Plant Physiol., 4, 349, (1929).
[21] Gane, R.—Journ. of Pamol., 13, 351, (1935).
[22] Gerhardt, F.—Plant Physiol., 1, 251, (1926).
[23] Hansen Elmer and Hartman Hennry.—Plant Physiol., 12, 441, (1937).
[24] Harvey, R. B.—Science n.s., 67, 421, (1928).
[25] ———————— Univ. Michigan, Agr. Expt. St. Bull., 247, (1928)
[26] Hartshorn, R.—Plant Physiol., 6, 467, (1931).
[27] Hibbard, R. P.—Michigan Sta. Tech. Bull., 104, (1930).

[28] Haynes, D.—Ann. Bot., XXXIX, 77, (1925).

[29] Kidd, F.—Nature, 135, 3409, (1935).

[30] Kidd, F and West, C.—Great Britain Sci. and Ind. Res. Food Invest. Bd. Report, (1924, 1925, 1933 and 1935).

[31] Kidd, F., West, C., Griffiths, D. G., and Potter, N. A.—Ann. Bot., N.S., 4, 1, (1940).

[32] Kar, B. K. and Banerji, H. K.—Nature, 144, 597, (1939).

[33] ─────────────────────── Current Sci., 9, 321, (1940).

[34] Lane, J. H. and Eynon, L.—Soc. Chem. Ind. Trans., 42, 327–377, (1935).

[35] Loomis and Shull.—Methods in Plant Physiology, (1937).

[36] Martin, W. E.—Plant Physiol., 11, 139, (1936).

[37] ───────── Bot. Gaz., 99, 42, (1937).

[38] Nelson, R. C.—Plant Physiol., 12, 1004, (1937).

[39] ─────────Ibid., 14, 817, (1939).

[40] ─────────Ibid., 15, 149, (1940).

[41] Rosa, T. J.—Proc. Amer. Soc. Hort. Science, 22, 315, (1926).

[42] Ranjan, S. and Khan, Z.—Journ. Ind. Bot. Soc., 13, 17, (1934).

[43] Regeimbal, L. O., Vacha, G. A., and Harvey, R. B.—Plant Physiol., 2, 357, (1927).

[44] Singh, B. N., Seshagiri, P. V. V., and Gupta, S. S.—Indian Jour. of Agr. Science, 7, 176, (1937).

[45] ──────────────────────────────── Ann. Bot., N.S., 1 ,311, (1937).

[46] Wolfe, H.—Bot. Gaz., 92, 337, (1931).

[47] Work, P.—Proc. Amer. Soc. Hort. Science, 25, 61, (1928).

[48] Archbold, H. K. and Widdowson, E. M.—Biochem. Jour., 25, 863, (1931).

VII. STUDIES ON THE GROWTH AND DEVELOPMENT OF JUTE (*CORCHORUS CAPSULARIS*) WITH SPECIAL REFERENCE TO (1) THE REQUIREMENT OF BORON DURING ITS LIFE-CYCLE AND (2) THE RELATION OF BORON TO THE 'DIEBACK' EFFECT

By B. K. PALIT

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The importance of trace elements in the nutrition and growth of plants has been emphasized by several investigators. And among them the utility of boron in the growth and development of plants has been the subject of many investigations. Among the investigators who carried out early the experiments on boron requirement of plants, the names of Brenchley [1] and Warington [9] in England and Sommer and Lipman [2] in America, need special mention. McMurtrey [3] has given a short summary of the work on boron requirement by plants. It has been noted that the use of this element leads to widely divergent results regarding the growth behaviour of different plants. In summarizing the work on boron requirement by plant Miller [4] observed recently that of all the plants that have been investigated, 'cotton has the highest boron requirement and cereals the lowest'. The literature on the use of boron to the growth behaviour of plants has been reviewed by Shive and Robbins [5]. Very recently the work on the requirement of boron by plants has been reviewed in *Nature* [6] under the caption 'Boron in Agricultural and Horticultural Practice'. It seems well established that if an element is lacking or present only in minute quantity or in excess in the nutrient solution the growth responses under these varying condition of supply will also be different.

It is probable that a particular plant will require a particular type of solution for optimum growth because plants vary in their tolerance to acidity or alkalinity [7].

Last year in the course of investigation of jute plants grown in pots filled with manured soil and kept in open it was noticed that many of the plants developed characteristic injury, the result being that the topmost bud leaves and a few other assimilating leaves shrivel and fall off and the stem withers, become brown at first and ultimately blacken; the injury afterwards spreads downwards. This type of injury commonly designated as 'dieback' was not observed in plants grown on a trial plot. It was supposed that due to heavy monsoon rains last year some of the inorganic salts essential for plant growth were washed away from the soil in the pots. This effect was also observed in case of plants grown last year in sand culture which will be described later. Similar injury reported in literature has been traced to (1) the deficiency of

boron [8, 9], potassium [10] or moisture [11] in the soil, (2) the addition of ammonical fertilizers [12], or (3) frost [13].

In the present paper is reported a series of investigations with jute plants undertaken with a view to study their growth and other characteristics when raised under various conditions in the open field, in pots containing composted soil and in sand culture, the last two were grown in a glass house. The results of the investigations is given in the following sections. In §1 is reported the results of investigations on the effect of trace elements chiefly boron on the growth of jute, in §2 is given the diurnal growth records of jute plants in composted soil and in sand culture. It was found that plants grown in pots in the glass house, both those in composted soil, as also others in sand cultures, showed certain characteristic 'dieback' effect. In §3 is reported the investigations which were carried out with a view to finding out whether the 'dieback' effect was due to deficiency in certain trace elements. In the case of the plants grown in the open field, a small number of plants were observed to suffer temporarily from similar dieback effect; and in §4 is given an account of investigation similar to that given in §3 with plants grown on the open field. In §5 is discussed the results of the investigations reported in §§1–4, and an account is given of the tentative conclusion arrived at on the origin of the dieback disease in jute. Further investigation will be necessary before a definite conclusion is obtained.

§1. Effect of the trace element Boron on the growth of Jute plants

Materials and Methods

Jute (*Corchorus capsularis*) seeds were obtained from the Royal Agri-Horticultural Society at Alipore, Calcutta, on May 16th, 1940. Seeds were selected for uniformity of size and weight. Experiments were carried out in sand and solution culture, but detailed results reported here have been taken from the sand culture experiments as it is easier to grow plants under this method.

The sand used was a special silver sand of fine grains obtained from glass factory through the courtesy of Prof. N. C. Nag. The sand was first dried in a hot sterilizer at a temperature of 80–85°C. for 2 hours. Next it was digested with 5% HCl and thoroughly washed and subsequently treated with 5% NH_4OH and finally washed with several changes of distilled water until the reaction of the water is neutral to boron.

The vessels used were earthenware pots 6″ high and 6″ diameter with an opening at the bottom of it. The outer and inner sides of the vessel were coated with high melting point paraffin (Kahlbaum make).

The chemicals that have been used in the experiment were of purest grade Kahlbaum reagents. Spectroscopic examination of the reagents, however, was not performed. Distilled water was always used in preparing the solutions.

The solution used in these culture experiments was Hoaglands solution (5, 5, 2 and 1 millimole per liter respectively of $Ca(NO_3)_2$, KNO_3, $MgSO_4$, and KH_2PO_4). To each liter of this solution was added 0·05 p.p.m. of zinc and 0·05 p.p.m. of manganese and occasionally 0·02 p.p.m. of copper. And iron was added in the form of 0·5% ferric citrate at the rate of 1 c.c. per liter as required by the plant during its growth.

Experiments were performed in a glass house having suitable arrangements for free ventilation. The temperature of the glass house was found to vary through about 8°C. in 24 hours. The plants were exposed to light under the ordinary conditions of day and night.

Special means were devised to irrigate the plants with culture solutions. A 1-liter separating funnel was charged with the solution and the rate of flow of solution was regulated to be such as to drain a liter of liquid in 24 hours. The drops of solution as it falls on a glass funnel specially prepared, entrain slugs of air which are released into the plant pot through an opening into the wood used as a top cover of the plant pot. The cover is used to prevent the growth of algae and other parasitic growths. The solution as it enters the plant pot at first saturates the sand and the excess is drained into a pyrex 1 liter beaker. The plants were flushed twice a week with distilled water to maintain the balance of the nutrient media. The nutrient solution prepared and used for the first day were also made use of on next two successive days. The pots were then flushed with distilled water, and the whole procedure is repeated.

The jute seeds were soaked in distilled water for 10–15 minutes, followed by the sowing of 4 to 5 seeds in each pot on sterilized sand in distilled water, on the 24th May, 1940. Germination took place on the next day. When the seedlings were ten days old only two of the plants out of 4 or 5 were allowed to remain in each pot. The seedlings were irrigated with Hoagland's solution, as has been described previously, excepting that boron of six different concentrations varying from 0 p.p.m. to 10 p.p.m. were added, as boric acid to the solution.

Seedlings were also grown in pots in manured soil side by side with the solution culture and sand culture under the identical conditions of light, temperature and humidity. In all sets of experiments samples grown in composted soil were taken as control.

Experiment I.—The plants were grown under this condition for 42 days before first harvesting. The first set of five plants was harvested on the 16th July of the same year. After harvesting the roots were washed free from sand. Measurement of elongation of stem and petiole were then taken. Determination of the area of the surfaces of the leaves were made by means of a planimeter. After taking all the measurements the plants were divided into two fractions, viz. (*a*) the tops, and (*b*) the roots. Fresh weights were taken and the fractions were placed in a drying oven at 80–85°C. for three days.

Dry weights were next taken and the data obtained from them are incorporated in table 1.

Plants receiving no boron were fairly healthy. Only the leaves of the plants were not as green as those of the plants receiving boron. Plants receiving concentrations of boron beginning from 0·01 p.p.m. to 0·5 p.p.m. were quite healthy, the leaves were rather dark green. But with concentrations 0·1 p.p.m. of boron the growth was found to be maximum, even greater than those grown on the composted soil. Plants receiving 1 p.p.m. and 10 p.p.m. boron showed distinct toxicity symptoms; the leaves became spotted turning completely yellow in colour and were deeply cupped on the under surface. Total growth was much reduced, the apical stems in some cases died and the axillary buds that developed were all yellowish in colour. Roots were very poorly developed.

Table 1 gives the effect of varying amount of boron concentration on the growth and development of jute plants.

TABLE 1

Nature of solution	Average of five plants						
	Total height in cms.	Total area of assimilating cells in sq. cms.	Total length of petiole in cms.	Fresh weight of plants in grams		Dry weight of plants in grams	
				Tops	Roots	Tops	Roots
No boron ..	33·25	175·40	33·7	4·09	0·52	0·49	0·11
0·01 p.p.m. boron ..	50·7	318·85	48·3	8·38	1·54	1·03	0·17
0·1 p.p.m. boron ..	73·7	645·76	87·0	19·87	2 82	2·37	0·43
0·5 p.p.m. boron .	53·0	325·33	48·9	8·24	1·10	0·99	0·15
1·0 p.p.m. boron ..	24·7	119·67	20·9	2 19	0 41	0·26	0·07
10·0 p.p.m. boron ..	24·0	82·83	18·2	1·76	0·41	0·23	0·06
Control in composted soil ..	54·2	407·42	57·1	10·53	1·44	1·22	0·17

The time of growth of these plants, recorded in the above table, is 42 days. Seeds were sown on the 24th May, 1940, and harvested on the 16th July of the same year.

Fig. 1 (plate XVI) gives the photographs of jute plants grown with different concentration of boron in the nutrient solution and the results embodied in table 1 are represented graphically in fig. 2.

From the curve it will be seen that the maximum growth takes place with culture solution containing 0·1 p.p.m. of boron. It appears probable

that the optimum growth would take place at some concentration between 0·01 and 0·1 p.p.m. of boron.
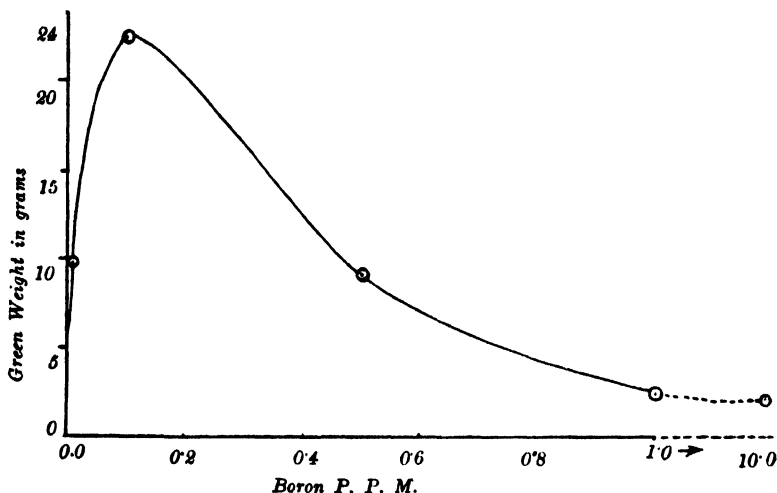

FIG. 2. Graphical representation of the green weight per 5 entire (top+root) jute plants, supplied with various concentrations of boron, 16th July, 1940, 42 days from the planting of seed.

Experiment II.—Another set of 10 plants were allowed to grow to maturity and when a sufficient number of flowers began to appear they were harvested. This set of plants were germinated on 30th May, 1940, two plants as usual were allowed to remain in each pot. The surplus number of plants were weeded out on the 10th June and from this day plants were irrigated with nutrient media till maturity. The plants were harvested on the 18th September of the same year. After harvesting the roots were washed free from sand. Measurement of height of stem were then taken and determination of the fresh weight and dry weight of plants were made. It is to be noted here that plants receiving the highest concentration of boron, viz. 10 p.p.m., died before attaining maturity and the plants receiving 1 p.p.m. of boron also exhibited signs of decay; all the leaves had fallen. Only the stem with bud leaves remained. For the plants grown with 10 p.p.m. boron only the height and dry weight of plants are given because other measurements are not possible. Plants receiving no boron remained alive to the end of the experiment. As will be seen from table 2, the general growth of the plants raised in no boron culture solution was much reduced as compared to those of the plants receiving 0·01 p.p.m., 0·1 p.p.m. and 0·5 p.p.m. of boron.

The summary of results obtained with varying level of boron concentration on the growth and development of jute plants is seen detailed in table 2.

TABLE 2

Nature of solutions	Average of ten plants				
	Total height in cms.	Fresh weight in grams		Dry weight in grams	
		Tops	Roots	Tops	Roots
No boron	90·5	15·60	2·83	1·70	0·32
0·01 p.p.m. boron	163·0	47·74	4·99	6·15	0·74
0·1 p.p.m. boron	171·0	51·98	4·52	8·24	0·89
0·5 p.p.m. boron	119·0	24·45	3·55	2·73	0·45
1·0 p.p.m. boron	56·5	6·88	1·63	0·67	0·18
10·0 p.p.m. boron	39·5	nil	nil	0·30	0·08
Control in composted soil	212	101·11	9·21	20·20	2·62

The time of growth of these plants is from the 30th May, 1940, when the seeds were sown, to the 18th of September, 1940, when the plants were harvested.

The measurement of elongation of stem and area of assimilating cells of leaves were left out of this table (cf. table 1) on account of the production of many side branches. Side branches contained many not fully matured leaves and to avoid confusion and error in determining the number and area of the fully assimilating leaves these measurements were omitted.

Another noticeable feature is that all the plants grown in pots in manured soil as control for experiments I and II died on account of aphis infestation. Consequently, all data regarding control experiments given in table 2 were taken from plants grown in the open field in manured soil and sown on the same date. Though these plants were of the same age they showed much better growth compared to the plants grown in the best nutrient media and under the highly favourable laboratory condition. Repetition and extension of the tenure of the experiment gave results consistent with those obtained previously and flowers did not appear when boron was withheld whereas flowers appeared normally in the case of plants supplied with boron. Much difficulty, however, was experienced in keeping the plants growing freely up to the flowering stage and many plants suffered severely with aphis infestation. Treatment of the upper part of the jute plant with Bordeaux mixture solution gave some relief to the plants.

§2. DIURNAL GROWTH RECORD OF JUTE TAKEN IN SUMMER 1939

Experiment III.—This experiment was started in summer 1939 to study the diurnal growth of jute plants grown in pots under different manurial

conditions. A preliminary investigation with plants grown in composted soil and in sand culture was carried out during the month of July. The manure used in the case of soil culture was nitrogeneous dung manure and the solution that was used in the sand culture experiments was Knop's solution plus 1 p.p.m. of boron and 0·05 p.p.m. of manganese and zinc; the salts used in the last two cases were sulphate and in the former case boron in the form of boric acid was used. Iron was added in the form of ferrous sulphate at the rate of 1 c.c. of 0·5% ferrous sulphate per liter. Plants grown in soil was watered with tap water.

Two growth recorders for measuring records of growth were utilized in obtaining diurnal records from each of the samples of plants grown in (a) sand culture, and (b) composted soil. Special device as were used in recording the longitudinal growth of the stem of Helianthus [15] was employed in this case. Magnification of the writers was adjusted to be low, here being 2½ times. The plate moved laterally through 6 inches in 24 hours. The interval between two successive dots is 15 minutes.

The longitudinal growth measurements of samples grown in sand culture were continued under normal varying condition of light and temperature. For convenience only a typical curve is reproduced here (fig. 3, plate XVII) which gives an idea about the nature of the growth on a particular day, though the rate of growth was different in successive days.

Thirteen such records were obtained in 13 successive days and the total growth during 24 hours are given in the following table:

TABLE 3

Days of observation	1	2	3	4	5	6	7	8	9	10	11	12	13
Total elongation in mm. during 24 hrs. ..	6	10	13	18	25	29	32	34	29	20	11	5	0

This table shows the total elongation of growth for 24 hours for 13 successive days in *sand culture*.

Similar measurements of samples of plants grown on manured soil were recorded under the identical conditions of light and temperature as that of plants grown in sand culture. Here also a typical curve showing elongation in longitudinal growth is reproduced in fig. 4 (plate XVII).

Eighteen such records were obtained on 18 successive days. The data of the results are given in table 4.

9

TABLE 4

Days of observation ..	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Total elongation in mm. during 24 hrs. ..	5	9	13	17	23	27	30	34	38	40	42	44	40	36	30	21	9	2

The above table shows the total diurnal growth of jute for 24 hours for 18 successive days in *manured soil*.

It can be seen from the figs. 3 and 4 given in plate XVII, that there is a regular periodicity in the growth of jute grown in sand culture which, however, was not noticed in the curve obtained with manured soil. But both the plants showed 'dieback' effect and failed to survive up to maturity. It was observed at this time that plants grown in the open field did not show this effect.

§3.   STUDY OF 'DIEBACK' EFFECT

A.   'Dieback' effect in plants grown in sand culture

TABLE 5

Concentration of boron	0 p.p.m.	·01 p.p.m.	·1 p.p.m.	·5 p.p.m.	1 p.p.m.	10 p.p.m.
Time of appearance of 'dieback' effect	24/7/40	19/7/40	14/7	7/7	3/8	3/8
Age of the plants in days when the effect appeared in them ..	60	55	50	43	70	70

Table 5 gives the time of appearance of 'dieback' effect on plants grown in sand culture and as recorded in experiment. In table 6, given below, is recorded the various treatments to which another set of plants grown in sand culture were subjected in order to get rid of the 'dieback' effect.

TABLE 6

Nature of treatment	Result
1. Uprooted plants and roots aerated	Nil.
2. Top dressing with strong iron citrate solution ..	Tissues scorched.
3. Sand flushed with distilled water immediately on the appearance of 'dieback' effect.	Plants appear to recover, apex of stem began to turn green from base to tip.

With regard to experiment II, table 6, it is to be noted that the flushing with water is in addition to the normal flushing of the sand after every three days.

B.   Dieback in plants grown in open field

Jute plants have been grown in a plot of land measuring about 30 ft. by 30 ft. in the Institute garden in Calcutta.   The soil used was a slightly alkaline one.   The land was thoroughly cultivated and the soil was manured with nitrogeneous dung manure.   After receiving a rainfall of about 2 inches the land was ploughed again.   The seeds obtained from the former source were broadcasted on the 30th May, 1940.   Seeds as usual germinated on the next day.   The first thinning out of the plants was made after a fortnight when the plants attained a height of 4-5 inches.   It was noticed that some of the plants began to show 'dieback' effect about the middle of July, 1940. To ascertain the cause of this the following investigation was carried out.

§4.   PLANTS GROWN ON THE OPEN FIELD

Experiment IV.—A small portion of the land was isolated from the main field on which jute has been grown by digging trenches.   This portion was further divided into 4 smaller plots each measuring 9 sq. ft.   Around each of these plots trenches were dug out 1 ft. in depth and 6 inches in breadth to isolate every plot from its neighbours.   For convenience 4 plots are designated as P, Q, R and S respectively.   The total number of plants in these plots were 55, 52, 51 and 48 respectively.

PLOT P.—TABLE 7

	Height of plants in cms.					Nature of treatment	Results
	A	B	C	D	E		
On commence-ment of treatment (14th July)..	62	102	100	60	76	1 p.p.m.boron	
At the end of treatment (7th Aug.)..	died	204	167	111	131	Same	Recovery

PLOT Q.—TABLE 8

	Height of plants in cms.					Nature of treatment	Results
	A	B	C	D	E		
On commence-ment of treatment (14th July)..	70	46	94	85	70	1 c.c. of 0·5% ferric citrate per liter of tap water.	
At the end of treatment (7th Aug.)..	157	164	200	164	121	Same	Recovery

PLOT R.—TABLE 9

	Height of plants in cms.					Nature of treatment	Results
	A	B	C	D	E		
On commence-ment of treatment (14th July)..	70	91	73	56	79	1 p.p.m. boron and 0·5% ferric citrate per liter of tap water.	
At the end of treatment (7th Aug.)..	133	184	163	153	157	Same	Recovery

PLOT S.—TABLE 10

	Height of plants in cms.					Nature of treatment	Results
	A	B	C	D	E		
On commence-ment of treatment (14th July)..	80	64	61	81	87	Only tap water	
At the end of treatment (7th Aug.)..	236	139	119	210	176	Same	Recovery

It appears that under all the various modes of treatment the 'dieback' effect disappeared. It was also observed that the plants in the main plot not subject to any special treatment had recovered from the 'dieback' effect.

Later on, on tabulating the rainfall which occurred during the period of growth of the jute plants it was seen that the appearance of the 'dieback' effect

was associated with the period of comparative drought, and the subsequent disappearance of the effect coincided with the ending of the period of drought. The point is discussed in detail in §5.

TABLE 11

No. of observation	Date of observation	Total rainfall in inches
1	3rd week of June	4·18
2	4th ,,	3·95
3	1st week of July	2·64
4	2nd ,,	1·55
5	3rd ,,	1·70
6	4th ,,	1·13
7	1st week of August	2·34
8	2nd ,,	1·75
9	3rd ,,	2·86
10	4th ,,	2·37
11	1st week of September	2·57

Table 11, given above, shows the amount of rainfall which occurred during each week beginning from the 3rd week of June to the 1st week of September, 1940.
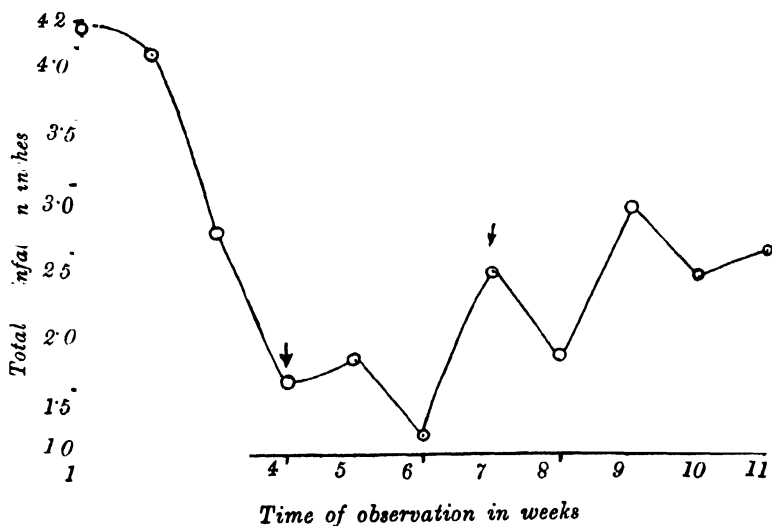

FIG. 5.   Graph showing the total rainfall per week beginning from the 3rd week of June to the 1st week of September, 1940.

On analyzing the curves given in fig. 5 it will be seen that the rainfall decreased after the 1st week of July and this state continued up to the end of this month after which there was again an increase in rainfall in the 1st week of August.

§5. DISCUSSION

The salient fact disclosed by the experiments I and II reported in this paper is that boron as an element is necessary for the growth of jute. On the basis of the height, area of assimilating cells of leaves, fresh weight and dry weight of plants, as shown in table 1, the optimum range of boron concentrations for jute grown under conditions of these experiments lies between 0·01 p.p.m. to 0·1 p.p.m. Plants grown in solution to which 0·5 p.p.m. of boron was added, were fairly healthy, but above this value, viz. 1·0 p.p.m. and 10·0 p.p.m. of boron concentration, appear to be toxic to plants. Plants grown to maturity in sand cultures under the conditions of experiment II, have produced flowers with nutrient solutions containing boron concentrations of 0·01 p.p.m., 0·1 p.p.m. and 0·5 p.p.m. Flowers, however, were not seen to appear in plants receiving no boron with the nutrient. Elongation of stems and roots of plants in no boron nutrient was small but did not cease altogether. On attaining the age of 100 days or more when they had attained maturity, these plants had a more or less barren appearance, with no leaves, no side branches, and the colour of the apical bud leaves turned white. Plants receiving 10·0 p.p.m. boron in the nutrient died early, on their attaining the age of 50–70 days. Plants growing in the nutrient to which 1·0 p.p.m. of boron had been added survived longer, viz. 80–100 days, but in a very unhealthy condition; all the leaves fell off, the main stem only remained somehow alive in a moribund condition and produced no flowers.

Thus it is seen that the use of the element boron is beneficial within certain limits to the growth and development of jute, though it is not altogether impossible to grow less vigorous plants without boron. No test, however, was made by us to find out whether there was any trace of boron present in the salts used for preparing the nutrient solution, nor any examination was made to determine whether any boron was present in the plants grown with no boron nutrient solution. Brenchley and Warington [14] observed that certain plants, viz. pea, barley and candy tuft, could be grown to maturity including flowering and fruit development without the addition of boron in the nutrient solution, as shown by spectroscopic examination. They also observed that from normal growth and development of soyabean, scarlet runner bean, crimson, red, yellow and white clover the element boron is essential. They admitted, however, that the difference in the growth behaviour of the two series of plants is the degree of concentration of boron used in the two different groups, for by using still lower concentration of boron in the former case, this difference practically vanishes. Moreover, they remarked

that seed of the plants of the former groups may contain enough boron for their development.

The similarity in growth under varying conditions of supply of nutrient, viz. with nutrient solution in case of plants grown in sand culture and with tap water in the case of plants grown in manured soil, is shown not only in the growth responses of respective plants in 24 hours of growth but also by the close similarity of their growth on successive days. These might lead to the conclusion that the solution culture in question is a satisfactory replica of soil, but observing, however, the patterns of the growth curves figs. 3 and 4, described in experiment III, some difference appears, viz. there is a regular periodicity in all the records obtained from plants grown in sand culture, the number of pulses varies between 8–10 in 24 hours, but no such periodicity in growth was noticed in soil grown plants. The pulsatory nature of growth elongation was also observed in a paper on the 'Periodic variation in longitudinal diametric growth of stem in Helianthus'[15]. This periodicity in growth response might have some relation in the movement of sap. The reason for the absence of similar periodicity in growth shown by plants grown on soil is not quite clear.

The 'dieback' effect reported in experiment IV of this paper has been observed in plants grown under three different conditions, viz. (1) in sand culture experiments, (2) in soil grown controls, and (3) in the open field. Though the conditions of growth are widely different, the effect disclosed by these plants are remarkably the same. The causes which lead to this effect in different cases, as has already been discussed in the introduction of this paper, are many and varied. The experiments I and II on sand culture were conducted from the latter part of May to the end of September, 1940, and the solution being a balanced one the question of excess of nitrogen did not arise. It will be noticed also that sufficient potassium was present in the nutrient solution, to which in the different trials various concentrations of boron in the shape of boric acid were added. It should be noted here that iron was added in the form of iron citrate.

It has been found also that plants grown in sand culture to which no boron was added, exhibited the same symptoms of dieback. But one thing should be particularly noticed that plants receiving concentrations of boron in the nutrient necessary for optimum growth were the first to show signs of this effect. This clearly shows that there must be some difficulty in the utilization of mineral salts by the plants. It might have been either that (1) the original concentration was changed, or (2) the pH of the solution may have gone more to the acid side thereby interfering with the supply of sufficient nutrient to the roots. As has already been described in experiment IV, the effect seemed to disappear by flushing the sand with distilled water only. It was found in experiments I and II, that the pH of the solution in some series varied from 5·5 to 2·5 on using the same solution for irrigating the sand on three successive days. As stated before in between the application of

freshly made solution the sand was flushed one day with distilled water. From the experience gathered it appears probable that the effect would not have appeared if daily fresh nutrient solutions were used.

The results obtained with plants grown in the open field in four different plots seemed to indicate that supply of additional amount of boron, iron or a mixture of boron and iron did not materially help the growth of the plants in any way as can be found by a comparison of the data of the tables given in experiment IV, with those which were supplied only with water. It might have been the case here that the concentration of salts present in the soil solution was not favourable for the absorption by the roots of the plants in question. Moreover, the appearance of and recovery from the 'dieback' effect in the case of non-treated plants'grown in the open field is associated in a very striking manner with the corresponding rainfall during the period. The 'dieback' effect appeared on the field grown plants about the middle of July when there was comparatively less rainfall and began to disappear at the beginning of the month of August when there was more rains. This also strongly favours the suggestion that either (1) the concentration of the salt in the soil water present in the vicinity of the roots must have undergone some variation, or (2) the pH of the soil solution is changed. From a review of all our observations it appears probable that the 'dieback' effect is due to a change in the pH value of the soil, caused in the case of plants grown in sand culture to the use of the same nutrient solution on three successive days, and in the case of plants grown in the trial plot to a temporary failure of the monsoon rains. This point will be specially tested in experiments planned for the next rains.

SUMMARY

On the basis of the measurement of height, assimilating area of the leaves, fresh weight and dry weight of plants, the optimum range of boron concentrations for jute grown in sand culture with nutrient solution lies between 0·01 p.p.m. to 0·1 p.p.m. Concentration above and below these values were found to be less effective.

The rates of diurnal growth of plants grown in 1939 July in sand culture and in composted soil show a similarity. But the patterns of the curves obtained with plants grown under two different conditions are different. Periodicity in growth is observed in the case of plants grown in sand culture, but no such periodicity, however, is observed in soil grown plants.

The 'dieback' effect is found to occur in plants grown under three different conditions, viz. (1) in sand culture experiments, (2) in soil grown controls in pots, and (3) in the open field. While the present investigations do not permit any definite conclusion to be drawn, as to the causes of dieback observed in jute plants, the following causes are probable. In the case of plants grown in sand due to the same solution being used on three successive days leading to a change in the pH of the solution. In the case of the plants grown in the

open field due to a temporary failure of the monsoon leading to (i) a change in the pH of the soil solution near the roots of the growing plants, or (ii) an increase in the concentration of the salt in the soil solution which reduces the intake of the soil solution by the roots of the jute plant.   Further investigations to test these points are contemplated.

In conclusion I beg to express my grateful thanks to Dr. D. M. Bose, the Director of the Institute, for the keen interest he has shown and the valuable criticism he offered to me throughout these investigations.

REFERENCES

1 Brenchley, Winifred, E.—On the action of certain compounds of zinc, arsenic, and boron on the growth of plants. Ann. Bot., 28: 283–301. 1914.

2 Sommer, A. L. and Lipman, C. B.—Evidence on the indispensable nature of zinc and boron for higher green plants. Plant Physiol., 1: 231–249. 1926.

3 McMurtrey, J. E.—Distinctive plant symptoms caused by deficiency of any one of the chemical elements essential for normal development. Bot. Rev., 4: 183–203. 1938.

4 Miller, E. C.—Plant Physiology. McGraw-Hill Book Co., Inc., p. 341. 1938.

5 Shive, J. W. and Robbins, W. R.—Trace element nutrition.   Ann. Rev. Biochem., VIII: 510–514. 1939.

6 Boron in Agricultural and Horticultural Practice.   Nature, Vol. 145, p. 766.   1940.

7 Hoagland, D. R. and Arnon, D. J.—The water culture method for growing plants without soil.   Ann. Report of Smithsonian Inst., U.S.A., Washington, p. 477.   1939.

8 McMurtrey, J. E.—The effect of boron deficiency on the growth of tobacco plants in aerated and unaerated solutions. J. Agr. Res., 38: 371–380. 1929.

9 Warington, K.—The effect of boric acid and borax on broad bean and certain other plants.   Ann. Bot., 37: 629–672.   1923.

10 Hart, C. E.—Potassium deficiency in sugarcane. Bot. Gaz., 88: 229–261. 1929.

11 Heald, F. D.—Manual of Plant Diseases. McGraw-Hill Book Co., Inc., p. 99. 1933.

12 Floyd, B. F.—Dieback or Exanthema of citrus trees. Fla. Agr. Exp. Sta. Bull., 140: 1–31. 1917.

13 Heald, F. D.—Manual of Plant Diseases. McGraw-Hill Book Co., Inc., p. 169. 1933.

14 Brenchley, Winifred E., and Warington, Katherine. --The rôle of boron in the growth of plants.   Ann. Bot., 41: 167–187.   1927.

15 Das, S. C. and Palit, B. K.—Periodic variation in longitudinal and diametric growth of stem in Helianthus.   Trans. Bose Res. Inst., IX: 35–57.   1933-34.

FIG. 1. Jute grown from seeds in sand culture with and without addition of
boric acid. The control is marked (*a*) on the extreme left. The plants
designated as (*b*), (*c*), (*d*), (*e*) and (*f*) received respectively 0·01 p p.m.,
10·0 p p m., 0 5 p p.m., 0·1 p p m. and 1·0 p.p m. of boron. The
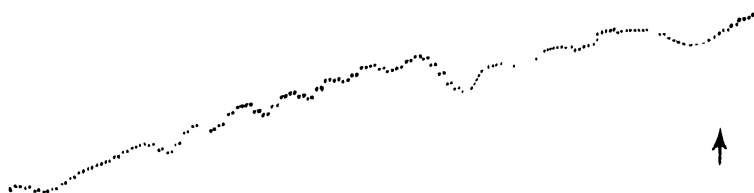plants were irrigated by the drip and drain method.

FIG. 3.   Record of longitudinal growth of jute grown in sand culture for 24
          hours   The abscissa represents the hours of day and night and the
          ordinate, the growth elongation.   The growth is indicated in the
          direction of arrow as an up-curve.
             Successive dots are at intervals of 15 minutes.

FIG. 4.   Record of longitudinal growth of jute grown in composted soil for 24
          hours   The growth is indicated in the direction of arrow as an up-
          curve.   Dot interval 15 minutes.

VIII. THE DISPERSION OF SUPERSONIC WAVES IN WATER

By A. K. Dutta and B. B. Ghose

The dispersion of supersonic waves in gases have been definitely established for some time now and this led to various investigations into the field of dispersion of supersonic waves in liquids. But although various attempts have been made by various workers [1-10] the problem has not been definitely settled this way or that. It is due to the fact that the dispersion effect, if it is there, is of a very small amount and requires very great experimental accuracy. The investigation acquires all the more importance, because in many of the liquids there is a large amount of absorption, which cannot be accounted for by the classical Kirschoff-Stokes law of absorption. Theoretical attempts towards correlation of the dispersion and absorption in liquids have been attempted by Kneser [11] and by Dutta and Ghose [12] on the basis of the relaxation theory. In the latter paper it has been obtained that associated with the high absorption in many liquids, one expects a measurable amount of dispersion also, in the high frequency region of 10^8 cycles and more, but in the particular case of water one does not expect any amount of dispersion, which is due to the fact that the main Raman frequencies lie very high. It has been surmised that one could measure the dispersion of supersonic waves in liquids also, provided one could work in the high frequency region of more than 10^8 cycles. But, unfortunately this region is yet beyond the scope of experimental technique. That in the comparatively lower frequency region one measures an appreciable absorption and no dispersion is due to the fact that the amount of dispersion falls off much more rapidly than absorption, as explained in detail in the paper referred to.

The case of water is, however, unique. Here, although, we do not expect any dispersion due to the relaxation mechanism, akin to gases, it was observed by one of us,[6] that compared with Toluol, Xylol, Dekanil, etc., the velocity of supersonic waves increased with frequency in the case of water. Further measurements on the dispersion of supersonic waves in water has been undertaken by many workers, and most of them have not been able to measure any dispersion effect in the same region of frequency. In view of these contradictory results, the following investigation of the measurement of the dispersion of supersonic waves in water was undertaken again.

Accurate Method for Dispersion of Supersonic Waves

Before going into the details of experimental arrangement, it is desirable to discuss the possible methods of measurement critically, so that one can

understand what particular cares should be taken to measure the dispersion in liquids. The most convenient and accurate method for measuring the velocity of supersonic waves in liquids is, of course, the well-known diffraction method of Debye-Sears. The arrangements should be adjusted such that the diffraction lines on the photographic plates are very fine. For this it is very often advisable to get only the 1st order spectra with low intensity, specially with higher frequencies. The photographing camera should be of very large focal length (preferably, about 1 meter) so that the separation is large and the relative accuracy in measurement very high. Secondly, the temperature must be accurately (correct up to less than ·1 degree) known, at the time of taking the photograph, and the same temperature must be maintained while photographing the low frequency as well as the high frequency spectra, of the same liquid. This requires a very sensitive controlling device as due to the higher absorption, the temperature of the liquid in the case of higher frequency rises much more rapidly and attains a much higher temperature than in the case of the lower frequency region.

In order, therefore, to eliminate such elaborate controlling system, many workers have taken the photographs with very rapid plates and strong light source, and have taken their photographs immediately after applying the supersonic field on the liquid, so that a rise in temperature of the liquid can be disregarded. The temperature, however, was correctly read at the time of the exposures. From the point of view of the measurement of temperature, this method should give results of the desired accuracy. But to photograph a field, immediately on generating the oscillations, involves one in another difficulty. It is well known that immediately after generating oscillations in a high frequency circuit, the frequency fluctuates appreciably, and one can expect a steady oscillation only after allowing it to run for nearly half an hour. The immediate photographing method has thus a great drawback. From the point of view of the accuracy of measurement of frequency one should wait for some time after starting the oscillations.

Lastly, the measurement of the frequency of the oscillator with an error of less than 1 in 1,000 requires a wavemeter of very good make and special calibrations. Ordinary, fairly good wavemeters can read accurately only up to 1 in 100 and is evidently of no use in experiments on dispersion.

From these considerations one can suggest only two methods which can measure the supersonic dispersion accurately.

(1) Putting the supersonic field on, one should allow the liquid to be heated up to its equilibrium state, thus allowing the oscillator to attain the steady frequency. The frequency should now be read with a precision wavemeter and the photograph recorded with a camera of near about one meter focal length. With another frequency again, exactly the same equilibrium temperature should be obtained after allowing the generator to run for some time. This inevitably requires a thermostat with adjustable control. The frequency and the photograph should be recorded as before.

(2) According to the second method one can eliminate the use of the wavemeter, by comparing the diffraction patterns of one liquid with the other at two exactly corresponding frequencies. This is possible by keeping the two circuits well shielded and undisturbed, the oscillating quartz itself being put in a separate inner vessel with a window, and having a permanent liquid inside it up to a definite height. As the results will show one can keep the steady frequencies constant up to about one part in ten thousand, so long as the circuit remains the same. The results, however, will show a ratio of the dispersion in two liquids and by trying different liquids, one can estimate the absolute amount of dispersion. In the following experiment, as in the case of the previous experiment by one of us, this method has been followed.

Experimental arrangement

The velocity of supersonic waves in the liquids was obtained by the well-known diffraction method. Two transmitters were used, one for the comparatively lower frequency of near about 10^6 cycles and the other for 10^7 cycles and more. In the low frequency oscillator a Hartley circuit was used with the Telefunken tube of type RS.282. The quartz oscillator had a fundamental frequency of 10^6 cycles and its harmonic, giving a frequency of nearly 3×10^6 was used for the lower frequency. The quartz oscillator was directly coupled with the oscillating set. In the high frequency oscillator we used a push-pull circuit with the Telefunken tubes RS.276. A separate quartz plate of fundamental frequency of 10^7 per second was utilized, the harmonic 3×10^7 (approx.) being always used. The high frequency quartz oscillator was indirectly coupled with the coil of the oscillating set. The transmitter was shielded with wire nettings which were earthed.

The experimental liquid was placed inside a rectangular glass vessel of 14 cm. $\times$ 7 cm. $\times$ $9\frac{1}{2}$ cm., whose sides are optically plane. Inside the glass vessel, close to one of its sides, was placed another metallic vessel having a water-tight mica window and containing xylol up to a definite level. The crystals were kept immersed in xylol hanging vertically from supports resting on the walls of the glass vessel. The sound waves excited in the inner vessel were transmitted to the outside liquid through the mica window. The whole system was placed inside a water bath contained in a rectangular double jacketed vessel having two parallel glass windows. This vessel was fitted with a stirrer and a thermo-electrical-relay working in conjunction with a heating coil dipped into the water. The coil was heated by means of low voltage A.C. current. The thermostat vessel is also provided with a platform on which the glass vessel was placed. The glass vessel was kept always in the same position by means of fixed uprights attached to the platform. The temperature of the liquid inside the glass vessel was noted by means of a Beckman's thermometer reading up to 1/100°C.

The optical system consisted of a source of the green light of mercury, filtered from the mercury arc (in the case of the high frequency spectrum the

arc was not filtered as it diminished the intensity unnecessarily), and a spectro-scopic arrangement for photographing the spectra. The camera had a focal length of 95 cm.

The photographed lines were measured by means of a Hilger Comparator reading correctly upto 10^{-4} cm.

During the experiment, sufficient time was allowed to enable a steady temperature to be reached, and by adjusting the control, the temperature of any liquid was kept constant within 0·01°C. at both the frequencies. Precau-tions were also taken to see that identical conditions regarding the geometric positions of connecting wires were maintained.

For each liquid at any definite temperature and frequency, three spectra were recorded and then the set was adjusted for another frequency or another liquid. This procedure was repeated a number of times for each pair of liquids on successive days. Thus by comparing the spectra at two frequencies in the same liquid, we obtain from one set of measurements, the ratio of the frequencies if we consider the velocity in this liquid to remain constant. For, the separation between any two lines of a given order

$$d \propto \frac{\text{Frequency}}{\text{Velocity}}$$

at the same temperature and under the same optical condition. If, however, in any liquid, the velocity changes by a constant factor, on changing from one frequency to another frequency, the ratio of distances (for the higher and lower frequency) will correspondingly change. An increase in velocity would show a decrease in the ratio compared to that in the standard liquid in which the velocity does not change with frequency. But although the ratio of distances 'd' might change in different liquids according to the dispersion involved in these liquids, the ratio in any liquid should remain constant under ideal conditions provided the frequencies remained constant. The change in the ratio for different liquids gives the amount of dispersion.

Photographs were taken with two liquids at the two frequencies under ideal thermal and electrical conditions as described above and the ratio of the distances 'd' were found out in the case of two liquids. It was observed, further, that so long as the sets were not disturbed the ratio for each liquid, generally, remained constant within 2 in 10,000 on successive days. A number of constant ratios were obtained for each pair of liquids. The temperatures for any liquid were not identical on successive days but were kept constant each day for the two frequencies. Thus we can consider that ideal conditions were maintained and that the frequencies remain constant.

The results are tabulated in Table 1. It has been obtained that the ratio of the distances 'd' in water diminished by 1·5±0·2 in 1,000 compared to the ratios obtained in the case of Toluol, Dekanil and Xylol. The temperature of the liquids remained near about 33° on different days. This means that in water at about 33°C. there is a dispersion of 1·5±0·2 in 1,000, provided we

TABLE I

Date	To be added to 32°·0C.		Low frequency			High frequency			
	Temperature read by Beckman Thermometer		Liquid	Order of the spectra	Mean distance	Order of the spectra	Mean distance	Ratio of distances for the same order	Dispersion
	L.F.	H.F.							
18-8-40	1·32	1·31	water	3	6·018	1	19·710	9·825	1·3‰
	1·38	1·37	toluol	5	12·106	1	23·820	9·838	
22-8-40	1·73	1·73	water	3	6·018	1	19·705	9·823	
	1·96	1·95	toluol	5	12·104	1	23·820	9·837	1·4‰
23-8-40	1·35	1·36	water	3	6·022	1	19·713	9·820	
	1·36	1·35	toluol	5	12·097	1	23·800	9·836	1·6‰
28-8-40	1·45	1·45	water	2	4·011	1	19·705	9·825	
	1·78	1·77	toluol	5	12·100	1	23·810	9·839	1·4‰
29-8-40	1·54	1·54	water	3	6·016	1	19·708	9·827	
	1·64	1·63	toluol	5	12·085	1	23·790	9·842	1·5‰
22-10-40	0·76	0·76	water	2	4·010	1	19·799*	9·864	
	0·78	0·78	xylol	4	9·536	1	23·550	9·878	1·4‰
27-10-40	1·35	1·34	water	2	4·002	1	19·740	9·865	1·3‰
	1·56	1·56	xylol	2	4·780	1	23·600	9·878	
29-10-40	1·53	1·53	water	2	4·003	1	19·740	9·865	
	1·73	1·73	xylol	3	7·163	1	23·590	9·880	1·5‰
30-10-40	1·15	1·15	water	2	4·003	1	19·743	9·865	
	1·25	1·24	xylol	3	7·165	1	23·595	9·879	1·4‰
22-11-40	1·33	1·32	water	3	6·038	1	19·843	9·864	
	1·55	1·54	dekanil	4	8·985	1	22·190	9·879	1·5‰
23-11-40	1·65	1·66	water	3	6·030	1	19·820	9·861	
	1·96	1·95	dekanil	5	11·233	1	22·190	9·877	1·6‰
25-11-40	1·40	1·40	water	3	6·035	1	19·850	9·863	
	1·67	1·67	dekanil	5	11·230	1	22·180	9·877	1·4‰
27-11-40	1·60	1·61	water	3	6·033	1	19·850	9·864	
	1·95	1·95	dekanil	4	8·980	1	22·180	9·879	1·5‰
	To be added to 45°.								
1-12-40	2·51	2·50	water	2	3·965	1	19·507	9·839	
	2·45	2·46	dekanil	4	9·388	1	23·112	9·847	0·8‰

' Connecting wires for the high frequency crystal was readjusted.

assume that there is no dispersion in Toluol, Xylol and Dekanil. The basis of this assumption·is that the ratio remained constant in different types of liquids like Toluol, Xylol and Dekanil. Attempt was also made to find out the dispersion at a higher temperature. The bath was maintained at 47°C. and similar control as at 33°C. was applied. The ratio of the distances in water and Dekanil were compared. It was observed that the diminution of the ratio in the case of water came up to about 0·8 in 1,000. As the high frequency quartz oscillator was damaged, this result could not be confirmed by taking repeated measurements. However, the indication is that the dispersion of water diminished appreciably on going up to a temperature of about 47°C. In this connection the previous result of one of us is worth comparing. It was observed there, that at a temperature of 27°C. the amount of dispersion was $1·7\pm0·2$ in 1,000.

As a result of further experiments in this laboratory, it was found out that the dispersion of water diminished with increased concentration of $MgSO_4$ as a solute. These results will be published in due course.

Discussion of other results

In view of the fact that on repeating our previous experiment we obtain the same result as against the contradictory results of other workers, it is perhaps desirable to look into the works of other people somewhat critically. We would, however, discuss only those results that have been obtained by the diffraction method. Other methods, generally, do not attain the required accuracy. The diffraction method, with fairly good accuracy in other points as well, has been attempted by Bär,[7] Matossi [8] and Krishnan.[9] Bär, as also Krishnan, have eliminated the thermostat and have taken recourse to quick photography for eliminating the rise of temperature of the liquid. They were thus forced to take the photographs immediately on applying the supersonic field. As has been discussed previously in this paper, the oscillator generally fluctuates at the start and may vitiate the frequency measurement due to this fluctuation. It will be observed from measurements of Bär that in the case of Benzene the velocity fluctuates by 1 in 1,000.

Krishnan considers that he could come to no definite conclusion. He says, however, that within the limits of experimental error he could detect no dispersion.

In the work of Matossi,[8] at a higher temperature than the room temperature, namely 27°C. (where sufficient temp. control is necessary) he obtained results identical with that of one of us [6] previously. When he took his measurements at room temperature, he could not get the dispersion generally. It was observed again in case of strong coupling. It appears from his paper that in his experiments at room temperature, he also took recourse to immediate photography in the case of one of the liquids. This may entail irregularities. Besides, it is very difficult to control the temperature where it

is very near the room temperature, and from his table it appears that the temperature of the liquid is sometimes lower than the temperature of the bath, although constancy in different liquids have been maintained.

Theoretical standpoint

It is necessary to stress one point regarding the theoretical implications. Kneser,[13] has compared the dispersion found by one of us and the absorption measurements of other workers and has come to the conclusion that such a high dispersion is incompatible with relaxation theory. It has, however, also been shown by us [12] that according to the relaxation theory no dispersion is expected in the case of water. So that, we agree with Kneser that dispersion and absorption in water in the given frequency range is incompatible with the relaxation theory, although the high absorption in other liquids is not incompatible with the relaxation mechanism. It appears to us that as water is a unique liquid in many respects, its dispersion also must be explained by some other mechanism. This appears all the more probable after a study of the dispersion in the case of $MgSO_4$ solution (to be shortly published). It has been observed by Buss [14] and also by Bazulin [15] that absorption in the case of $MgSO_4$ solution is much higher than that of water and increase very rapidly with concentration. According to the relaxation theory, the corresponding dispersion in the case of $MgSO_4$ solution should be higher than that of water and should increase more and more with concentration. But experiments undertaken here, show that the dispersion of water diminishes appreciably with increasing concentration of solute. The final solution of the problem, however, requires further experimental work, which is being undertaken in this laboratory.

Our thanks are due to Prof. D. M. Bose for his keen interest and valuable discussions.

SUMMARY

Dispersion of supersonic waves in water has been confirmed. Different methods for the measurement of dispersion in liquids have been discussed critically.

REFERENCES

[1] Boyle, R. W. and Taylor, G. B.—Trans. Roy. Soc., 21, 79, 1927.
[2] Spakovsky, B.—Compt. Rendu. U.S.S.R., 3, 588, 1934 and 18, 169, 1938.
[3] Sorensen, C.—Ann. der. Physik., 26, 121, 1936.
[4] Parthasarathy, S.—Proc. Ind. Acad. Sci., 4A, 222, 1936.
[5] Hiedemann, E. and others.—Naturwiss., 24, 681, 1936.
[6] Dutta, A. K.—Phys. Zeits., 39, 186, 1938.
[7] Bär, R.—Proc. Ind. Acad. Sci., 8A, 289, 1938.
[8] Matossi, F.—Phys. Zeits., 40, 294, 1939.

[9] Krishnan, K. G.—Proc. Ind. Acad. Sci., 9A, 382, 1939.
[10] Richardson, E. G.—Proc. Phys. Soc., 52, 480, 1940.
[11] Kneser, H.—Ann. der. Physik., 32, 281, 1938.
[12] Dutta, A. K. and Ghose, B. B.—Trans. Bose Res. Inst., 13, 31, 1938.
[13] Kneser, H.—Phys. Zeits., 39, 800, 1938.
[14] Buss, W.—Ann. der. Physik., 33, 143, 1938.
[15] Bazulin, P.—Compt. Rendu. U.S.S.R., 19, 153, 1938.

IX. VEGETATIVE PROPAGATION OF MANGO PLANT FROM GOOTES (MARCOTTE) AND CUTTINGS BY TREATMENT WITH HIGH CONCENTRATION AUXIN

By A. GUHA-THAKURTA and B. K. DUTT

Among the various effects brought about by the application of auxin on plants the rôle of root formation may be considered to have immense practical importance. The study of the effect of auxin on root formation of commercially important plants was first undertaken by Cooper[1]. By the apical application of auxin he obtained evidences that root formation in lemon, fig, *Acalypha* and *Lantana*, is accelerated by the application of root forming substances. Later on Cooper[2] and also Hitchcock and Zimmerman[3] successfully grew roots in the cuttings of various plants by applying high concentration auxin at the base. Afterwards this practice of growing roots by the application of high concentration auxin has been successfully utilized by different workers in propagating horticultural plants of different varieties. Most of the investigators have reported that treatment with auxin increases the percentage of the number of cuttings rooted and the number of roots produced per cutting, as well as reduces the time necessary for rooting.

The mango is one of the most valuable garden plants of India. The usual method of propagating pure variety mango, in this country, is by inarching, using seedling stocks. This method has its manifold disadvantages and does not always give satisfactory results. The object of this investigation was to find out an easier and more suitable method of vegetative propagation of mango, other than the present one of inarching. The present paper records results of root formation and subsequent vegetative propagation in mango by the application of hetero-auxin to the gootes and cuttings of the plant.

Experiment 1.—The experiment with cuttings of mango was first undertaken in the month of July, 1939. Fifty cuttings were made from twigs of current and previous year's growth from a twenty-year old mango tree growing in the Institute garden. All the leaves excepting the uppermost three or four, were discarded from the cuttings. They were then divided in two lots and their basal portions were immersed in 0·5% and 1% solutions of indole acetic acid respectively, for twenty-four hours. They were then placed in sand, in outdoor frames covered with moist cloth having an automatic arrangement for constantly moistening the same. The cuttings in this condition looked fresh for about a month but after that they began to decay. No root was found to grow in any of the specimens. In this connection mention may be

made of the work of Sen[4] who also reported his not very successful attempt to induce root formation in mango cuttings by treatment with indole acetic acid and indole butyric acid.

Experiment 2.—Our next attempt was to grow roots on the gootes of mango, this process being considerably less laborious for plant propagation than by inarching. In our preliminary experiment we employed two-year old plants in the month of September, 1939. In this process a complete ring of bark about one inch wide in the hard wood portion was removed, and the epidermis of the bark just above the ring was slightly disturbed with a knife. The wound was thoroughly washed with water and dried and then a lanoline paste containing 1% indole acetic acid was applied round the stem above the ring to the extent of half an inch. It was allowed to remain in that condition for twenty-four hours after which it was dressed with coir only and kept moist. Control experiments were also undertaken in which pure lanoline was applied instead of indole acetic acid. Roots were found to form in the treated regions of the branches within six weeks after application of auxin. There was no rooting in the control gootes. The number of plants employed in this experiment was eight, four auxin treated and four control. The gootes were not transplanted, for when kept undressed for a certain time for taking photographs, the exposed root tips dried up. Simultaneously with the above experiment another set of gootes was made in the two-year old wood portion of a big mango tree of about sixteen years old, by application of auxin. In this case instead of simple coir dressing a lump of soil was used inside the coir; but in spite of regularly watering for two months no root was visible from outside in any of the gootes. When however the dressings were removed after a long time profuse callus formation was observed in all of them and two or three small thick roots were found to have formed in two of the gootes.

The above results of the preliminary experiment suggest that the age of the plant may have some influence on the root formation in mango gootes. Recent investigation of Thimann and Delisle[5] on the cuttings of Conifers and of some dicotyledonous trees also indicate that among the different factors which influence the rooting of cuttings the most important are the age of the tree, auxin treatment, and the part of the tree from which cuttings are taken. They have shown that the plants which under normal condition practically never root from cuttings fall into two groups—(1) plants rooting readily on treatment with auxin, though scarcely at all without, (2) those not rooting appreciably with any auxin treatment. In the latter the most important factor is the age of the tree from which the cutting is taken. Cuttings from young trees root readily on treatment with optimal auxin. Stoutmeyer[6] showed that in apple tree green wood stem cuttings from shoots in the juvenile phase rooted very easily. Poesch[7] observed in ornamental plants that in general greater response was secured from young cuttings, possibly because of the greater content of natural hormones in the younger tissues.

TABLE I

Effect of indole acetic acid on the root formation in the gootes of young and old mango plants

Age of the mother plant	Treatment	Number of specimens used	Root formation as evidenced by their appearance outside the dressing. Number of specimens rooted—				Number of plants established in the soil
			after 2 weeks	after 3 weeks	after 6 weeks	after 9 weeks	
Two years	1% indole acetic acid	15	10	5	..	..	12
	Pure lano-line ..	15	..	..	2	2	1
Three years	1% indole acetic acid	10	6	4	..	..	8
	Pure lano-line ..	10	..	..	1	1	..
Twenty years	1% indole acetic acid	10	..	..	..	2*	..
	Pure lano-line ..	10	..	..	..	..	..

* When the gootes were undressed after five months growth of some very small roots were observed in four other treated specimens.

With a view to understanding the influence of age of the plant on root formation in gootes and cuttings of mango the experiment was further continued in the following year, 1940.

Experiment 3.—Root formation in the gootes of young and old plants: Two- and three-year old unassorted mango seedlings were collected and cultivated in a plot in the Institute garden. The experiment was performed in the rainy season, in the month of July, with the idea that the moisture of the season might help root formation, as well as to avoid frequent watering with hand pump. The gootes were made in the two-year wood portion of the plants. In the plants where two branches were available two gootes were made in the same plant, otherwise, a single goote was made in the main stem. The ring-bark cutting and its treatment with auxin were done in the same way as described in experiment 2. The gootes were covered with a lump of soil and properly dressed with coir. Altogether twenty-five gootes were treated—fifteen in the two-year old plants and ten in the three-year old ones. An equal number of control gootes were also made which were treated with pure lanoline instead of auxin paste.

Similar experiments, as above, were also done on an old mango tree aged about twenty. Ten treated and ten control gootes were made in the two-year old twigs of the tree. The results of the experiments are summarized in table I.

It will be found in table I that all the twenty-five auxin treated gootes in two- and three-year old plants, gave out roots. Out of them sixteen rooted after two weeks and the rest after three weeks. These lots seem to have rooted much earlier in comparison with the previous year's experiment described in experiment 2. The present experiment was started in the month of July when monsoon breaks in Bengal, instead of September when the atmosphere is comparatively drier. This shows that the high moisture content of the atmosphere has some influence on the rooting. The rooted specimens were severed from the mother plant and transplanted in pots. Among the twenty-five plants five died after a few months and the rest have become established in the soil and are maintaining vigorous condition up to the present time.

Among the twenty-five control specimens from young plants only six produced roots insufficiently and after a considerably longer period, three after six weeks and three after nine weeks. These rooted specimens were also transplanted in pots but only one of them has survived up to the present time and the rest died within a few weeks.

Out of ten auxin treated gootes made in the twenty-year old tree, only two produced a few roots which could be seen outside the dressing, after such a long period as nine weeks; but these did not survive after transplantation in plots. Later, in December when the unsuccessful gootes were opened some very small roots were found in four more treated specimens.

Experiment 4.—Root formation in cuttings of young and old plants: The experiment with cuttings from young and old plants was also undertaken in the same season of the year along with experiment 3. The method of treatment of the cuttings in this experiment was different from that used in the experiment 1, in which the base of the cuttings were immersed in the water solution of auxin. In the present experiment a ring of bark from the twig in which cutting was to be made, was removed and treated with lanoline solutions of auxin similarly as described in experiments 2 and 3. After twenty-four hours the twig was severed from the mother plant at the lower end of the ring. All the leaves except the uppermost four, were discarded and the branch was placed in a slanting position in an earthen pot containing soil. Cuttings were made from two-, three- and twenty-year old plants. All the cuttings were made from one-year wood portion of the plants. The cuttings from the plants of the varying ages were separately treated in the following order:—

1. 1% indole acetic acid.
2. 3% indole acetic acid.
3. 1% Napthelene acetic acid.
4. 3% Napthelene acetic acid.
5. Pure lanoline without any auxin.

A set of six cuttings was used in each of the above experiments. The results of the experiments are briefly summarized below.

RESULTS OF EXPERIMENTS WITH CUTTINGS

Cuttings from old tree.—The cuttings of old tree, both auxin treated and untreated, did not live more than a month. After they were dead they were taken out of the soil for examination, if there was any root formation. No root formation was observed in any of them.

Cuttings from young plants (control).—The control cuttings from two- and three-year old plants lived for two to four months and after that they died; no root or any callus was found to form in any of them.

Effect of Napthelene acetic acid on cuttings of young plants.—The cuttings from young plants treated with 1 and 3% Napthelene acetic acid died after three to five months. No root formation was observed in these plants; in the case of 3% Napthelene acetic acid there was only slight callus formation in three specimens.

Effect of indole acetic acid on cuttings of young plants.—The cuttings from young plants treated with 1 and 3% indole acetic acid gave better results in root formation. Those treated with 1% indole acetic acid lived for four to six months. On examination after their death callus formation was found in three of them and in another two small roots, five and three centimeters in length, were found to have grown. The best result was obtained by the application of 3% indole acetic acid. Out of six, two died after five months without producing any root; the rest rooted vigorously in the soil. All the rooted cuttings, however, for some reason or other did not finally survive. Two of them died after six months in spite of growing sufficiently large number of roots. One of the specimens growing vigorously was taken out of the soil, after seven months, for taking photographs of root growth, which is shown in plate XVIII. The other is living in a flourishing condition and has had already four flushings of leaves, sufficiently indicating that the plant is established in the soil.

CONCLUSION

It is evident from the experiments that vegetative propagation of mango is possible from gootes and cuttings by treatment with optimal auxin. The experiment further shows that the effect of auxin on root formation in gootes and cuttings is influenced by the age of the mother plant. The gootes and cuttings responded readily to auxin treatment when the plant was young, whereas, those of the aged tree responded very meagrely with the same concentration of auxin.

Further attempt will, however, be made to investigate means of successfully raising plants from gootes and cuttings of aged trees.

SUMMARY

The investigation was undertaken to find out an easier and suitable method, other than inarching, for the vegetative propagation of mango.

All the gootes from two- and three-year old plants treated with 1% indole acetic acid rooted within two to three weeks and 80% of them finally established in the soil. Some of the control gootes from young plants of the same age also rooted insufficiently after a considerably longer period, such as six and nine weeks, and only one out of twenty-five such control gootes finally established in the soil.

In the twenty-year old plant some of the gootes rooted but very insufficiently by treatment with 1% indole acetic acid and no plant could be raised from them. No root was found to form in the untreated gootes of the aged tree.

In the cuttings of young plants roots were formed by the application of 1 and 3% indole acetic acid, the latter producing much better results; propagation of plant has been possible from such cutting treated with 3% indole acetic acid. The control cuttings from young plants and also those treated with 1 and 3% Napthelene acetic acid did not produce roots, though they looked quite fresh and did not die out before a long time, such as three to five months had elapsed.

Cuttings from twenty-year old tree whether treated with auxin or not did not form any root nor survived for more than a month.

Thanks are due to Dr. D. M. Bose for his helpful criticisms and constant encouragement throughout the work.

REFERENCES

[1] Cooper, W. C.—Hormones in relation to root formation on stem cuttings. Plant Physiol., 10: 789–794. 1935.

[2] ———— Transport of root forming hormone in woody cuttings. Plant Physiol., 11: 779–793. 1936.

[3] Hitchcock, A. E. and P. W. Zimmerman.—Effect of growth substances on rooting response of cuttings. Contrib. Boyce Thompson Inst., 8: 63–79. 1936.

[4] Sen, P. K.—A note on the effect of indole butyric and indole acetic acids on rooting of green wood cuttings with special reference to Lichi and Mango. Current Science, 8: 553–554. 1939.

[5] Thimann, K. V. and A. L. Delisle.—The vegetative propagation of difficult plants. Jour. Arnold Arboretum, 20 (1): 116–136. 1939.

[6] Stoutmeyer, V. T.—The vegetative growth phases of apple shoots and their relation to root formation. Proc. Iowas Acad. Sci., 44: 104–105. 1937 (1938).

[7] Poesch, G. H.—Effect of growth substance on the rooting of woody ornamental plants. Ohio State Bimonthly Bull., 191: 56–62. 1938.

Photograph of mango cutting, showing root formation by treatment with lanoline
solution of 3% indole acetic acid

Photograph taken after 7 months of treatment

X. ON THE VARIATION OF CARBOHYDRATES AND THEIR TRANSLOCATION IN THE JUTE PLANT (CORCHORUS CAPSULARIS, LINN.)

PART I

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Translocation of organic substances is admittedly of primary importance to the higher plants and as such has attracted attention for a long time. But the conclusion arrived at by the earlier investigations are not always consistent with those obtained in recent years. Moreover, it seems that we are still far off from any uniformity of conception as to the various processes involved in translocation for which further work is necessary.

For an exhaustive review of the researches carried out in translocation, the reader is referred to the publications of Crafts [2], Curtis [6], Mason and Phillis [14] and others. It will appear that for a fuller and more comprehensive idea about the phenomenon of translocation in a plant, one has got to determine not only the path taken up by a solute for its migration, but he should also take into consideration the method by which that solute can move about and the factors that can influence this movement. For the tissue concerned in the translocation of organic solutes, recent investigations strongly support the case of phloem and the older conception of xylem being the route for upward and phloem for the downward movement of organic solutes or that of xylem serving both the purposes [6,7], do not seem to be tenable any longer. The analytical work of Mason and his co-workers [11] suggests that the sieve tubes are the main channel of translocation and they even could establish a positive correlation between the percentage of sieve tubes in any zone of bark and the sucrose concentration in that zone. According to them sucrose is the form in which carbohydrates are translocated [19].

As to the means by which this movement of organic compounds is accomplished, opinions differ and as a result several hypotheses have been put forward from time to time [6]. Here mention may be made only of three of them to complete this short review. They are, (1) the Druckstromhypothese, expounded by Münch [16,17,18], (2) the Diffusion theory strongly advocated by Mason *et al* [11,14], and (3) the hypothesis proposed by Crafts [2,3,4,5]. A bare outline of the first two hypotheses mentioned above may be given in the words of Mason and Phillis [13]. According to them 'the essential feature of Druckstromhypothese is that the sieve tube does not play an active part in the movement of materials through it. Solution is driven under pressure through the

vacuole and the sieve pores which are assumed to be open. Energy is supplied in the leaf by the metabolic activity of the transition cells which accumulate solutes from the mesophyll, and maintain the turgor pressure gradient in the sieve tube system. Thus, as long as the turgor pressure gradient is maintained and the sieve pores remain open, transport should continue. The Diffusion theory comtemplates movement through stationary cytoplasm. Diffusion is activated in some way by the energy released in the respiration of the sieve tube. Solutes travel independently of one another and of water; and the rate and direction of movement of each solute are determined by its own gradient'. Although Druckstromhypothese has been supported by some investigators [2,1], Weevers and Westenberg [22], Curtis [6] and Mason, Maskell and Phillis [12] have criticized it adversely on various grounds which are summarized by Curtis in his monograph on translocation (6, p. 229). The hypothesis advocated by Crafts is more or less a modification of that of Münch and as such many weaknesses attendant upon the hypothesis of Münch apply equally well to that of Crafts. His idea of the free movement of organic materials in plants through the side and end walls of the sieve tubes and not restricted to flow through sieve pores only, has met with much opposition [20]. The Diffusion hypothesis, on the other hand, has got one advantage over others that it is supported by a series of experimental evidences collected by Mason, Maskell and Phillis in their work on the cotton plant. They have established certain relations in conformity with the diffusion hypothesis but their work also do not always give a satisfactory explanation of various phenomena observed, e.g., the rate at which sugar is transported. Thus it has been calculated that in cotton plant the diffusion constant of sugar in the sieve tube is about 40,000 times as great as the diffusion constant for sugar in a two per cent solution of sucrose in water and that it is almost identical with the diffusion constant for molecules of the size of the sucrose molecule diffusing in air. This situation is no doubt very perplexing and to meet it we shall require a further consignment of intensive work.

The purpose of this investigation was to see how far the observation made in other plants with regard to the translocation of carbohydrates stand good in case of the jute plant. The data presented here are preliminary in nature but it is hoped that they will lead to further critical investigations.

MATERIAL AND METHOD

The material employed in this investigation was collected from jute plants (Corchorus capsularis, Linn.) grown in the experimental plots of the Bose Institute, Calcutta. The plots and the plants underwent normal treatment according to the practices followed by the cultivators; the manure employed was cow-dung.

Sampling.—Sampling began when the plants were about six weeks old with an average height of six feet and was completed in ten days. As a

preliminary measure the plants in the plots were divided roughly into groups according to their height, and for each experiment samples were collected from plants of the same group. Usually four plants went into a sample. For bark and wood samples usually one foot of the stem of each plant from its first basal node was cut out carefully, made into smaller sizes and skinned to separate bark from the wood. A sample of leaf, on the other hand, consisted of twelve to twenty mature normal leaves taken in equal number from the selected plants, beginning from the fifth to the successive leaves down below. All parts of a leaf went into a sample. All samples were weighed separately for their fresh weights before being put into boiling alcohol and preserved for analysis. Another set of comparable samples supplied the dry weights. When the plants were ringed their stems were covered with black paper in the previous evening and ringing operations were performed in an hour, early on the next morning. Rings were one centimetre wide at a height of about fifteen inches from the soil and the exposed wood was covered with vaseline. In ringed series also one foot of the stem immediately above and another immediately below the ring supplied samples for bark and wood. A ring in a plant was more than three feet below the region which supplied the leaves for a sample. Control plants for sugar and dry weight in the ringed series also underwent similar treatment, except the ringing operations and the samples were collected from comparable regions of the plants. In a third series of experiment for the determination of the extent of local photosynthesis in a stem, bark and wood samples were collected from the two feet of the stem of each plant from the first basal node. In this experiment half the number of selected plants had their stems covered with black paper while the other half were left exposed to serve as control. Moist chambers were used whenever necessary to minimize the loss of moisture from the samples and the process of gathering of the plants from the plots, cutting, weighing, etc., were performed with a minimum loss of time. Dry weights of the samples were determined after repeated heating in a regulated oven, cooling and desiccation.

The carbohydrates estimated in these experiments were limited to a determination of the total and reducing sugars and starch present in the bark, wood and leaves of the jute plants. The method employed for sugar analysis was that advocated by Van der Plank [21], with a slight modification in the process of inversion where hydrochloric acid was used instead of invertase. Starch was determined by the method followed by Hanes [9] from the dried samples after extracting sugar from them.

Expression of results.—The basis of expression of the results is in terms of the residual dry weights of the samples, as advocated by Mason and Maskell [10], and the data are given out as mgr. sugar per gr. mean residual dry weight. The data for the moisture content of the samples plotted in figures are also calculated according to these authors (10, p. 200). The quantity of starch was calculated on the ratio of 0·6 mgr. of maltose to 1 mgr. of starch [9].

EXPERIMENTAL RESULTS

1. Diurnal Series.

Moisture content.—Diurnal variations in moisture content of leaf, bark and wood are shown in table I and Fig. 1. In the table the results are given

TABLE I

	Gr. moisture per 100 gr. dry weight			Gr. moisture per 100 gr. residual dry weight		
Hour	Leaf	Bark	Wood	Leaf	Bark	Wood
7-30 A.M.	389·4	511·4	395·7	575·8	636·2	506·9
10-30 ,,	338·7	538·2	404·0	508·1	594·6	411·9
1-30 P.M.	321·8	502·5	393·9	451·1	619·5	433·5
4-30 ,,	328·4	516·7	436·9	436·6	628·3	484·7
7-30 ,,	332·9	507·1	415·4	474·3	625·5	491·1
10-30 ,,	370·6	498·4	402·7	539·3	625·2	475·8
Mean ..	346 9	512·4	408·1	497·5	621·5	467·3

Table I.—Diurnal variation in moisture content of leaf, bark and wood of the jute plant expressed in terms of dry and residual dry weight of the samples.
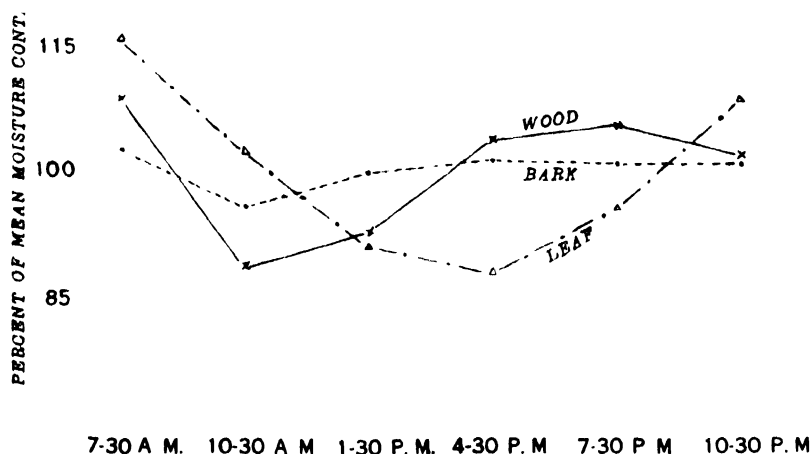
FIG. 1. Diurnal variation in moisture content of leaf, bark and wood.
(For calculation cf. Ref. 10, p. 200.)

in terms of both dry and residual dry weights of the samples. As expected the results in terms of dry weight are less than those in terms of the corresponding residual dry weight. The mean weight of the moisture content of leaf, it will be found, occupies an intermediate position between bark and wood when

expressed in terms of residual dry weight, while in terms of dry weight it occupies the lowest position. This may be due to the excess of carbohydrate present in leaf compared to that in bark or wood.

Moisture content of all the tissues exhibited variations during the course of a day: in general there was a fall from a higher level in the morning which was followed by a gradual rise towards the evening. Bark regained the loss very early and was followed by wood and then leaf. Fluctuation was most marked in leaf and least in bark, the latter maintaining a steady course from about 1-30 P.M. This may be due to the unavailability of the plenty of moisture present in the basal part of the stem, which supplied samples for wood and bark, to the transpiring leaves near the apex.

Sugar content.—Changes in the total sugar concentration of leaf, bark and wood in a day are shown in Fig. 2. The maximum quantity of total sugars

7-30 A. M.   10-30 A. M.   1-30 P. M.   4-30 P. M.   7-30 P. M.   10-30 P. M.
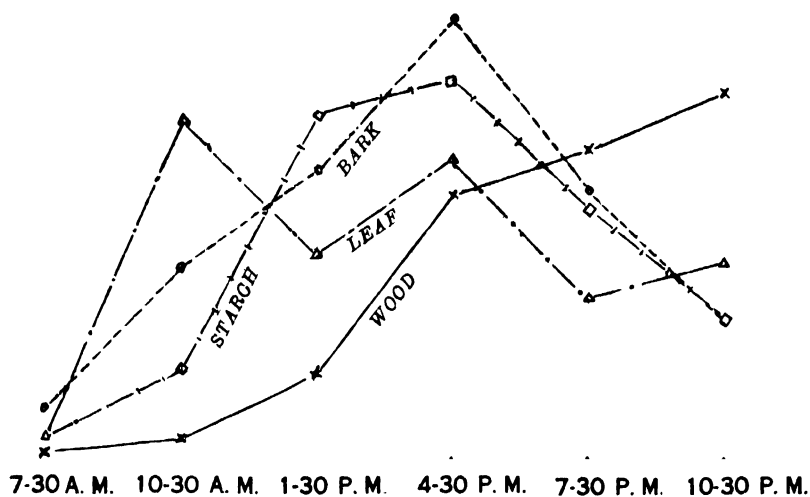
FIG. 2.   Concentration of total sugars and starch present in leaf, bark and wood.

(Sugars : Leaf—   1 divi. = 10   mgr., abscissa at 25 mgr.
Bark—   ,,   2·5 ,,   ,,   12 ,,
Wood—   ,,   1·0 ,,
Starch— ,,   4·0 ,,   )

was found in leaf and the minimum was present in the wood ; the mean values for leaf, bark and wood for the whole of the experimental period were 71·04, 24·88 and 3·96 mgr. respectively in terms of per gr. mean residual dry weight of the samples. In leaf and bark the quantity of sugar at first rose gradually to a maximum and then came down to a lower level in the evening. The course followed by leaf was rather fluctuating, but the maximum level attained by
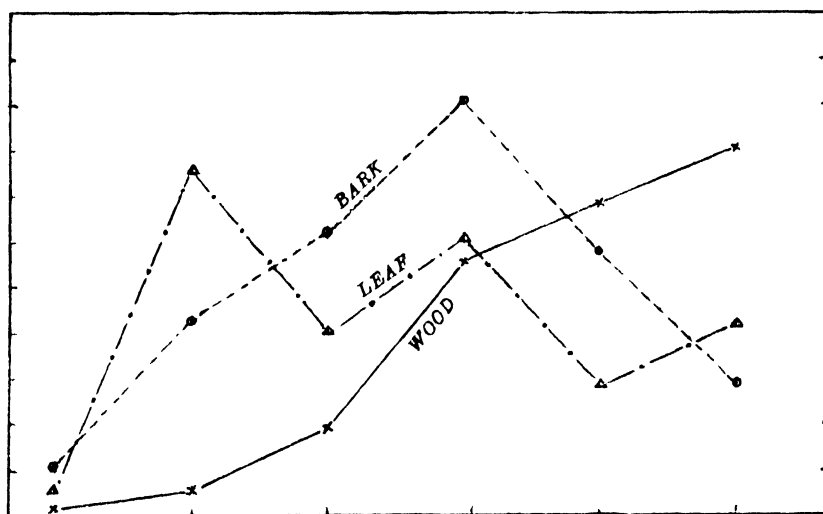
it was earlier in the day than that of bark (Fig. 2). In wood, on the other hand, the amount of total sugars rose steadily till the end of the experimental period,
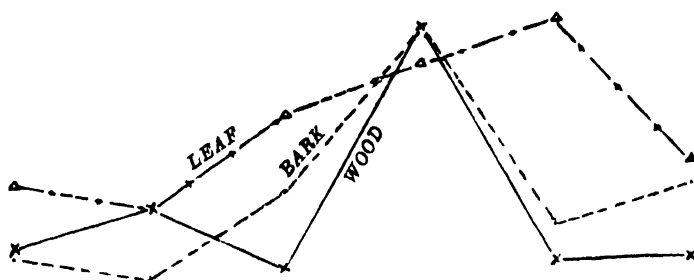
7-30 A. M.   10-30 A. M.   1-30 P. M.   4-30 P. M.   7-30 P. M.   10-30 P. M

FIG. 3.   Concentration of reducing sugars in leaf, bark and wood.

(Leaf—   1 divi. = 2,00 mgr.
Bark—   „   0·25 „
Wood—   „   0·05 „   )

7-30 A. M.   10-30 A. M.   1-30 P. M.   4-30 P. M.   7-30 P. M.   10-30 P. M.

FIG. 4.   Concentration of sucrose in leaf, bark and wood.   (For scale cf. Fig. 2.)

at first slowly and then at an accelerated rate which subsided considerably late in the evening.

The diurnal changes in reducing sugar and sucrose content of the three tissues mentioned above are shown in Figs. 3 and 4.   In these cases also the quantities of sugar gradually rose to a maximum as the day advanced and then came down to a lower level in the evening, except the sucrose content of wood which, like total sugars, maintained a rising course.   It will be seen that Fig. 4 is very similar to Fig. 2 and when the quantities of sugar present in each tissue are compared in these two figures, the difference, which is the reducing sugars, will be found to be very small in quantity, especially in wood and bark.   The mean quantities of sucrose present in leaf, bark and wood were 62·84, 24·24, 3·85 and that of reducing sugars were 8·2, 0·634 and 0·109 mgr. per gr. mean residual dry weight of the samples.   Reducing sugars in wood, unlike sucrose and total sugars, did not continue to rise but came down to a much lower level at the close of the experiment.

Starch.—Starch was present only in the leaf samples and it is conspicuous by its total absence in bark and wood, both in dried as well as in fresh material. The micro-chemical tests for starch indicate its presence only at the apical region of the stem.   In leaf the quantity of starch began in the early morning with a small quantity of 2·67 mgr. per gr. mean residual dry weight of the samples and reached the maximum of 31·58 mgr. at 4-30 P.M. after which it came down to 12·78 mgr. in the evening at 10-30 P.M.   The diurnal variations of starch at different period of the day are shown in Fig. 2.

2. *Ringed Series.*

Moisture content.—Changes in moisture content of the leaf, bark and wood samples of ringed and normal jute plants are given in table II and Figs. 5 and 6.   As in the previous case the quantity of moisture present in these samples also began to decrease with the advance of the day to be followed by an increase in the evening.   Here also the maximum fluctuation observed was in case of leaf.   In general the quantity of moisture present in ringed plants was higher than that of the normal (table II); in bark and wood the mean moisture content of the ringed plants were, above the ring, 108·6 and 111·4 and below the ring, 111·5 and 116·3% of the mean moisture content of the normal ones.   In case of leaf, however, the percentage was 97·3, although the quantity of moisture present in the first sample of leaves of the ringed plants was very high and perhaps this higher rate would have been maintained throughout the day, as in bark and wood, if the plants were left intact.   This is suggestive of the fact that ringing operations can interfere with the balance of moisture content of a plant and leaves are the first tissue to indicate the sign of moisture deficiency.   This was apparent from some more experiments where ringed plants were observed for several days.   In ringed plants, it will be observed, the decreasing rate of the moisture content was continued later than that of the normal and the lowest level attained by bark and wood was at 7 P.M. instead of about 3 P.M. as in the latter.   Leaf was, however, an exception to it and the

moisture content of the leaf samples of both ringed and normal plants attained the lowest level simultaneously at 3 P.M. (Figs. 5, 6).

TABLE II

Gr. moisture per 100 gr. residual dry weight						
Ringed				Normal		
Above				Above		
Hour	Leaf	Bark	Wood	Leaf	Bark	Wood
7 A.M.	595	572·4	481·3	511·6	482·5	424·6
11 ,,	452·2	498·2	458·6	501·6	475·2	383·6
3 P.M.	369·4	496·9	418·0	388·6	440·7	386·5
7 ,,	432·8	493·3	397·2	472·2	499·0	400·5
11 ,,	439·7	537·6	454·5	477·6	495·9	388·4
Mean ..	457·8	519 7	441·9	470·3	478·7	396·7
Below				Below		
7 A.M.	..	633·5	413·5	..	560·7	348·9
11 ,,	.	596·0	392·2	..	526·4	310·0
3 P.M.	.	580·6	359·4	..	515·0	327·7
7 ,,	.	573·8	321·6	..	561·9	337·1
11 ,,	..	662·7	438·1	..	567·4	330·6
Mean ..	..	609·3	384·9	..	546·3	330·9

Table II.—Moisture content of leaf, bark and wood of the ringed and normal jute plants.

Sugar content.—Changes in the quantity of total sugars present in leaf, bark and wood of the ringed and normal plants are given in Figs. 7, 8 and 9. It will be seen that the changes in the total sugar content of leaf of these two kinds of plants maintained a close level and the only deviation from the course was in case of the last sample of leaves collected from the ringed plants, where a higher quantity was registered. This is perhaps due to the effect of sampling, rather than to any stagnancy of sugar caused by a ring more than three feet below[10]. In the case of bark, however, there was a significant difference in the total sugar content of ringed and normal plants (Fig. 8). Thus it will be found that within eight hours the quantity of total sugars present in bark above a ring was higher than that in a comparable sample collected from the normal plants and this high rate continued to increase with time and attained still higher a level when the experiment came to a close. On the other hand, the quantity of total sugars present in bark below a ring did not exhibit any marked variation and maintained more or less a level course for the most of the period, whereas in normal stems the quantity of sugar in bark registered a marked rise in eight hours after which it began to decrease gradually towards

the evening (Fig. 8). The quantitative relationship existing between the sugar content of the samples of bark collected from ringed and normal plants is shown in table III. Attention may also be drawn to the quantities of sugar present in bark from the upper and lower parts of the normal stems, where it will be found that the quantity of sugar present in the upper part attained the maximum value earlier than that in the lower and also maintained a higher level throughout the experimental period (Fig. 8).
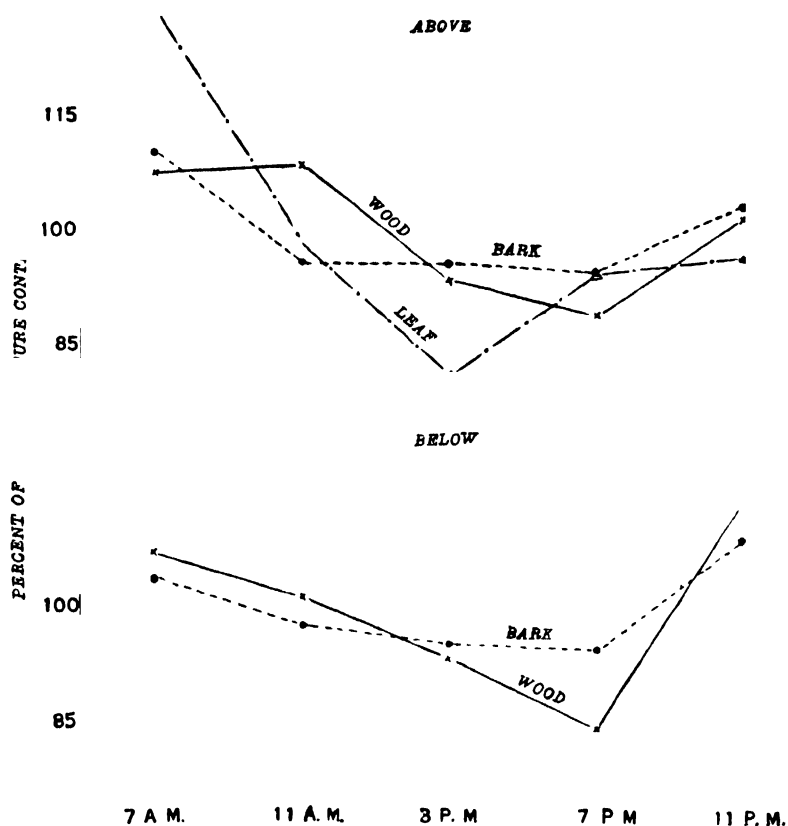

FIG. 5. Variation in moisture content of leaf, bark and wood of the ringed plants.

The total sugar content of wood from the ringed and normal plants are given in Fig. 9. Here also the quantities of sugar present in these two sets of plants did not exhibit any marked difference when the material was collected from the upper region of the stems, but in the lower region the changes in the quantity of sugar was very slow at the beginning of the experiment after which the increase in normal plants was much pronounced than that in the ringed

ones. Total quantity of sugar, it will be noticed, is small in the lower part of the wood than in the upper, both in normal as well as in ringed plants.

The changes in the quantity of sucrose in leaf, bark and wood of ringed and normal plants are shown in Figs. 10, 11 and 12 and those of reducing sugars in these tissues are shown in Figs. 13, 14 and 15 respectively. As in the previous case these changes in the sucrose content and the quantity of sucrose present in each tissue were very similar and close to those of total sugar
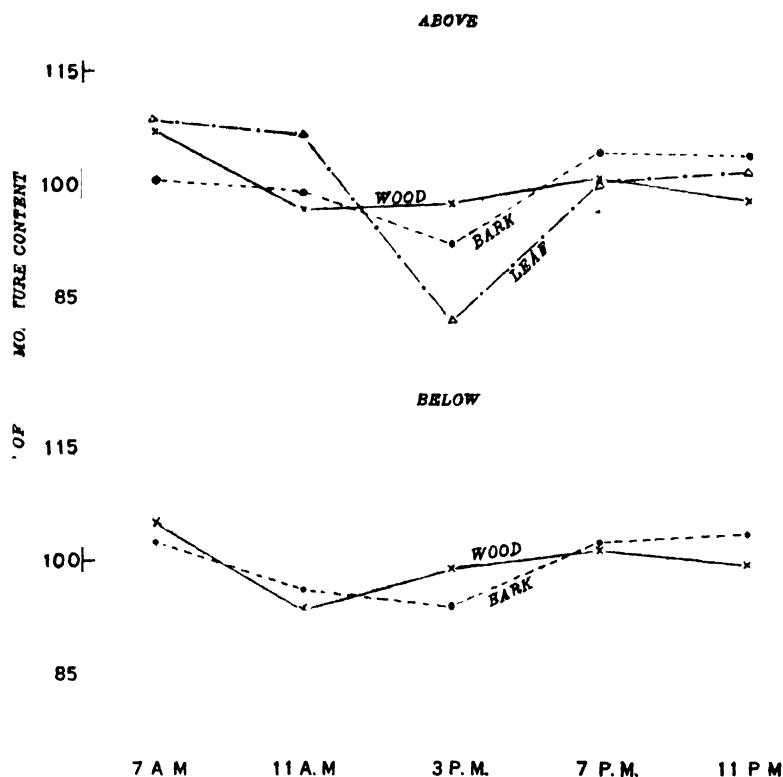
FIG. 6. Variation in moisture content of leaf, bark and wood of the normal (control) plants.

mentioned above and this is suggestive of the fact that sucrose is the main sugar present in these tissues. As regards reducing sugars it will be found that the changes in the sugar content were similar in all the tissues of both normal and ringed plants but there was difference in the quantities of sugar present in them, especially in wood where a higher rate was maintained by the ringed plants (Fig. 15). There was no marked difference in the sugar content of bark both from upper and lower region of the stem, but there was

a slight difference in case of leaf, which was due to a very high value obtained by the normal plants at 11 A.M (Fig. 13). In bark and wood samples of both normal and ringed plants the quantity of reducing sugars present in the lower region of the stem was very small in comparison to that in the corresponding upper region. This was particularly so in the samples of wood collected from
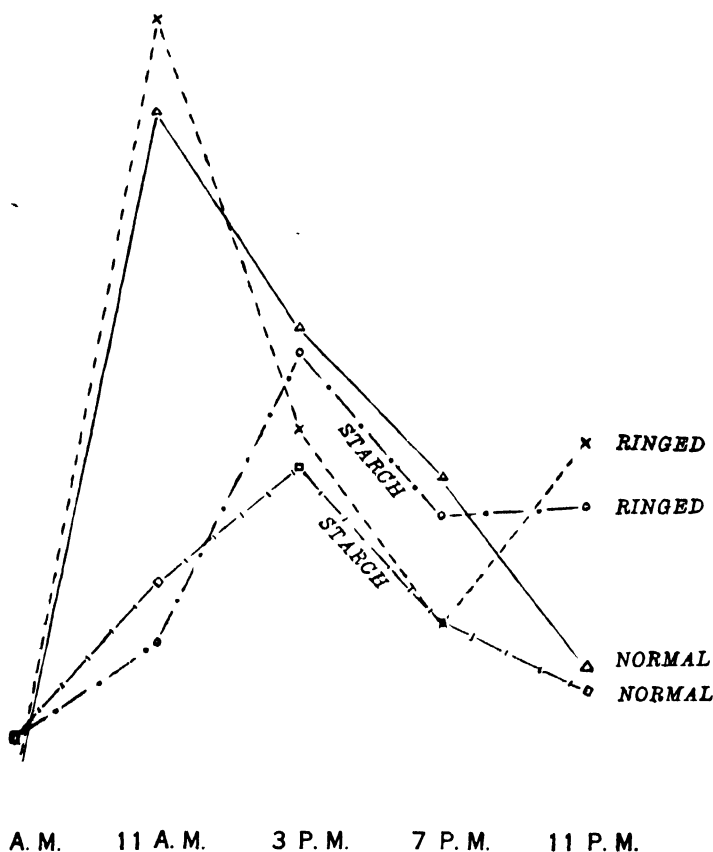

7 A. M.   11 A. M.   3 P. M.   7 P. M.   11 P. M.

FIG. 7. Concentration of total sugars and starch in leaf of the ringed and normal (control) plants.

(Sugar— 1 divi. = 10 mgr., abscissa at 40 mgr.
Starch—   ,,   4 ,,   )

the lower region, where especially in the morning, the quantity of reducing sugars was extremely small.

Starch.—In this experiment also the presence of starch was confined to the leaves and it was not formed in bark above a ring, although there was an excess of sugar available. The changes in the quantity of starch present

in the leaves of both normal and ringed plants are given in Fig. 7. There it
will be found that the data presented for these two sets of plants follow more
or less a definite pattern, but the quantity of starch present in the ringed plants
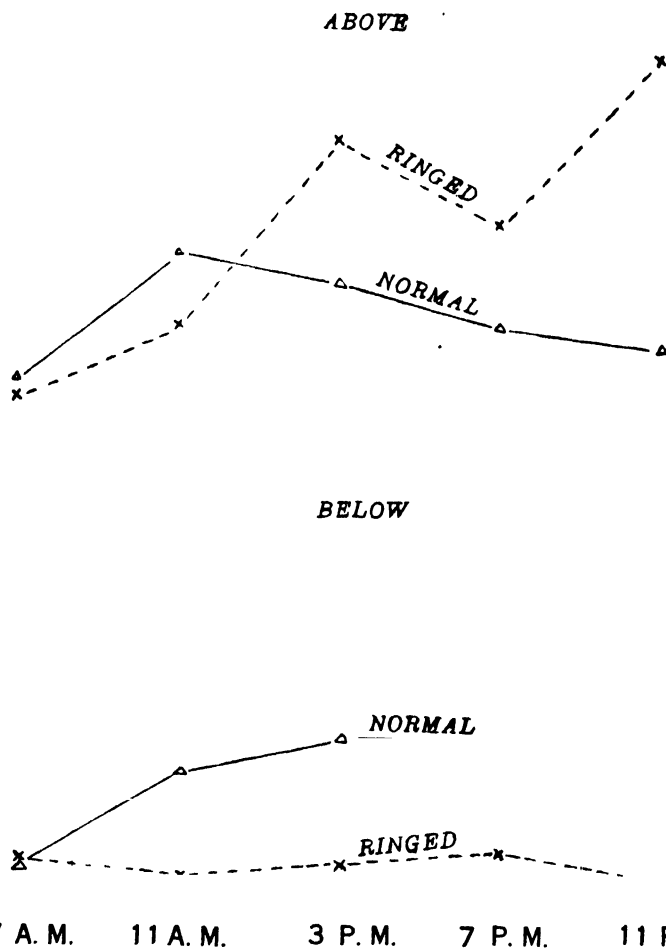

FIG. 8. Concentration of total sugars in bark of the ringed and normal (control) plants.
(1 divi. = 10 mgr.)

attained a higher level within eight hours from the beginning of the experiment
and this higher rate was maintained for the rest of the period.

3. *Covered and Uncovered Series.*

To determine the effect of covering the stem of a plant by black paper,
few samples were made at different time of the day from the stems of covered
and uncovered (normal) plants. It was thought that if any significant contri-
bution of sugar be made by the exposed bark of a plant, total sugar content

TABLE III

	Total sugars			Reducing sugars			Sucrose		
	Above			Above			Above		
Hour	Leaf	Bark	Wood	Leaf	Bark	Wood	Leaf	Bark	Wood
7 A.M.	100·3	82·8	106·8	156·4	166·1	113·3	97·2	81·8	106·4
11 ,,	110·4	67·1	374·1	65·8	78·6	216·7	181·5	67·2	378·9
3 P.M.	85·2	173·4	46·5	103·6	316·6	258·8	83·8	171·7	42·3
7 ,,	72·5	169·2	82·5	127·6	84·6	114·3	70·8	184·2	81·8
11 ,,	163·7	327·8	122·3	65·2	95·5	277·1	169·2	352·3	121·1
Mean ..	102·5	164·1	104·3	88·3	102·7	176·4	103·4	171·4	102·9
	Below			Below			Below		
7 A.M.		103·3	104·2	..	72·7	290	..	104·1	100·2
11 ,,	..	25·1	70·7	..	93·3	245·4	..	24·3	69·2
3 P.M.	.	26·2	15·2	..	260	119·5	..	23·9	13·4
7 ,,	..	34·2	35·5	.	82·2	135·9	..	33·3	35·2
11 ,,	..	26·6	..	..	126·2	..	..	24·5	..
Mean .	.	43·1	56·4	.	126·9	197·7	..	42	54·5

Table III.—Sugar content of the ringed jute plants as percentage of that of the normal.

in it should be higher than that in the covered one.   But the results set out below in table IV, however, are not sufficiently conclusive to corroborate any

TABLE IV

Mgr. sugar per gr. mean residual dry weight

	Covered						Uncovered					
	Bark			Wood			Bark			Wood		
Hour	Total sugars	Reducing sugars	Sucrose	Total sugars	Reducing sugars	Sucrose	Total sugars	Reducing sugars	Sucrose	Total sugars	Reducing sugars	Sucrose
6-45 A.M.	19·4	0·26	19·14	1·25	0·02	1·23	14·2	0·13	14·07	0·75	0·035	0·715
11-10 A.M.	24·6	0·24	24·36	1·62	0·035	1·585	34·1	0·36	33·74	1·62	0·30	1·32
2 P.M.	39·5	0·61	38·89	4·5	0·15	4·35	30·3	0·26	30·04	5·62	0 105	5·515
6 ,,	22·2	1·44	20·76	9·62	0·17	9·45	32·5	1·85	30·65	10·12	0·21	9·91
10 ,,	25·7	1·21	24·49	9·5	0·12	9·38	16·3	1·08	15·22	11·5	0·09	11·41
Mean	26·3	0·75	25·53	5·3	0·099	5·199	25·5	0·73	24·74	5·92	0·15	5·77

Table IV.—Sugar content of the stems of covered (with black paper) and uncovered (normal) jute plants.

such expectation. It is possible that the increase was perhaps too small to be detected by the method employed here.

DISCUSSION

From an analysis of the data given above it will be seen that all the sugars present in bark and leaf and the starch found in leaf of jute plants underwent considerable variations in the course of a day and the quantities of
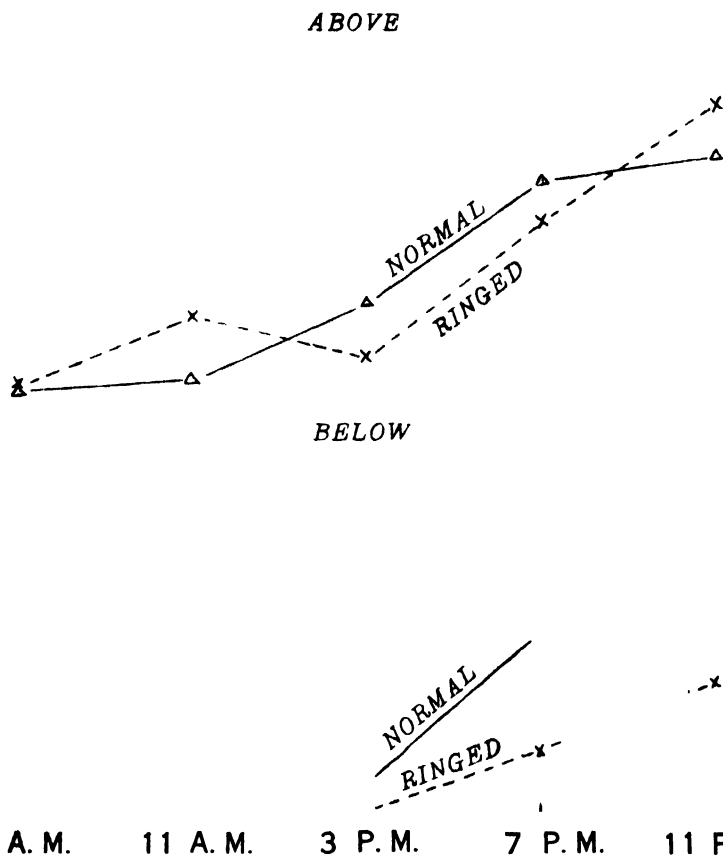
ABOVE

BELOW

FIG. 9.   Concentration of total sugars in wood of the ringed and normal (control) plants.
(1 divi. = 5 mgr.)

these substances in each of these tissues were usually small in the morning, which rose to a maximum at about the middle of the day and then fell again to a lower value (Figs. 2, 3, 4). This was also the case with the reducing sugars present in wood, but the total sugars or the sucrose content of this tissue, however, did not exhibit any such variation and instead there was a steady

rise to a maximum when the experiment came to an end (Figs. 2, 4). This observation was contrary to that made in other plants [10]. As regards quantity it will be found that the highest quantity of these sugars were present in leaf and the lowest in wood (Fig. 2) and when the quantity of starch present in leaf is taken into consideration it will be evident that the quantity of carbo-
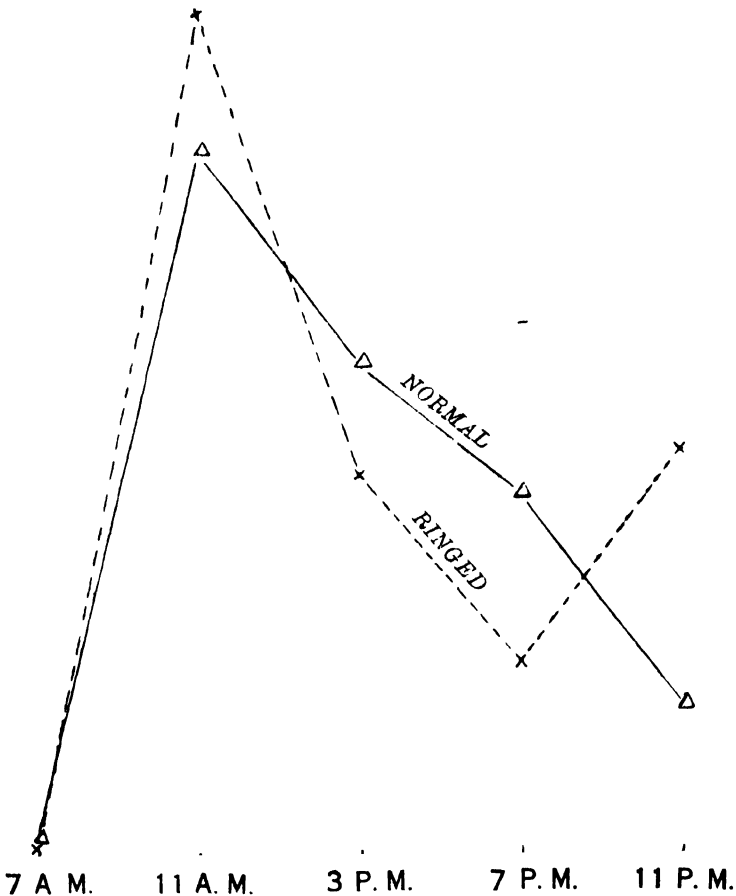

7 A M.   11 A. M.   3 P. M.   7 P. M.   11 P. M.

FIG. 10.   Concentration of sucrose in leaf of the ringed and normal (control) plants.
(1 divi. = 10 mgr., abscissa at 40 mgr.)

hydrates present in leaf was far greater in amount than any other tissue in the jute plant. So it becomes apparent that there is a downward gradient of sugar content from the leaf to the bark and the wood. When the quantities of sugar present in bark and wood collected from the two regions of the stems are taken into consideration, it will be found that there

is a positive gradient of sugar existing from the upper region to the lower
(Figs. 8, 9). Such a gradient has also been found to exist in cotton and
raspberry plants [10, 8]. In Fig. 2 it will also be seen that the quantity of
sugar attained its maximum level earlier in leaf than in bark [10]. The graphs
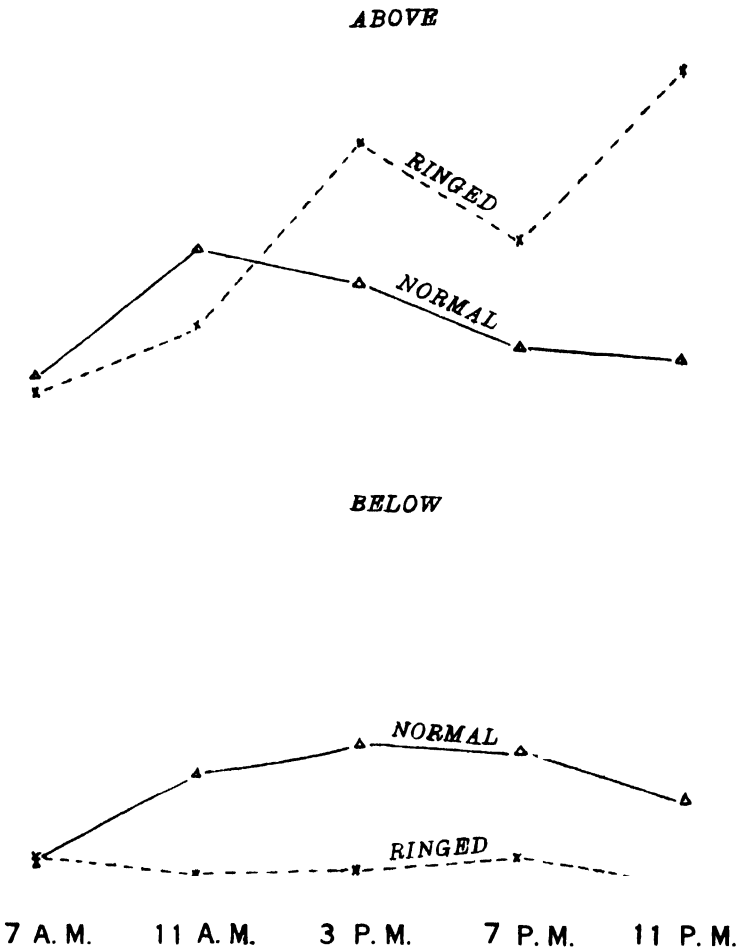plotted in this figure will also give an impression that the sugar manufactured

ABOVE

BELOW

7 A. M.   11 A. M.   3 P. M.   7 P. M.   11 P. M.

FIG. 11.   Concentration of sucrose in bark of the ringed and normal (control) plants.
(1 divi. = 10 mgr.)

in leaf migrated to bark and from there to wood, but certain considerations,
to be mentioned later, did not seem to lend a support to it.

In cotton plant Mason and Maskell [10] found that the quantity of sucrose
present in the stem was higher than that of reducing sugars, but in leaf, however,

the latter was in excess. They also found that the quantity of sucrose underwent maximum variations, wherefrom they came to the conclusion that sucrose is the form in which carbohydrates are translocated [10]. These two observations, however, were contradictory, for the fact that the theory of diffusion, which Mason and his co-workers advocate, warrants that there should be a higher concentration of the translocatory sugar at the source which was
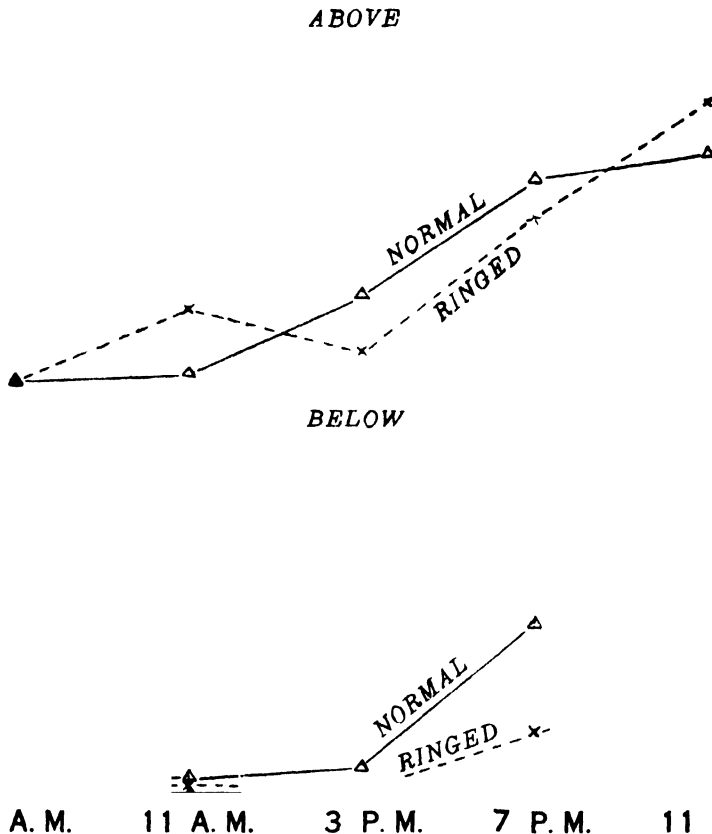

FIG. 12. Concentration of sucrose in wood of the ringed and normal (control) plants.
(1 divi. = 5 mgr.)

the leaf in their case. To meet this apparent contradiction Phillis and Mason [19] subsequently found that the sucrose concentration of the veins was much higher than that of mesophyll from which sugar was received. There was therefore a reverse gradient. Phillis and Mason thought that the companion cells and similar other cells were perhaps fitted up with a mechanism by which they can accumulate sucrose in the sieve tubes of the veins. In jute plant the

12

case was found to be slightly different. Here the quantity of sucrose in a whole leaf was highest and until further investigations are undertaken it is not possible to decide if at all the mesophyll contains a lower amount of sucrose in the jute plant. From the quantity of sucrose present in this plant and from the variations exhibited by sucrose it becomes apparent that probably it is in this form sugars are translocated.
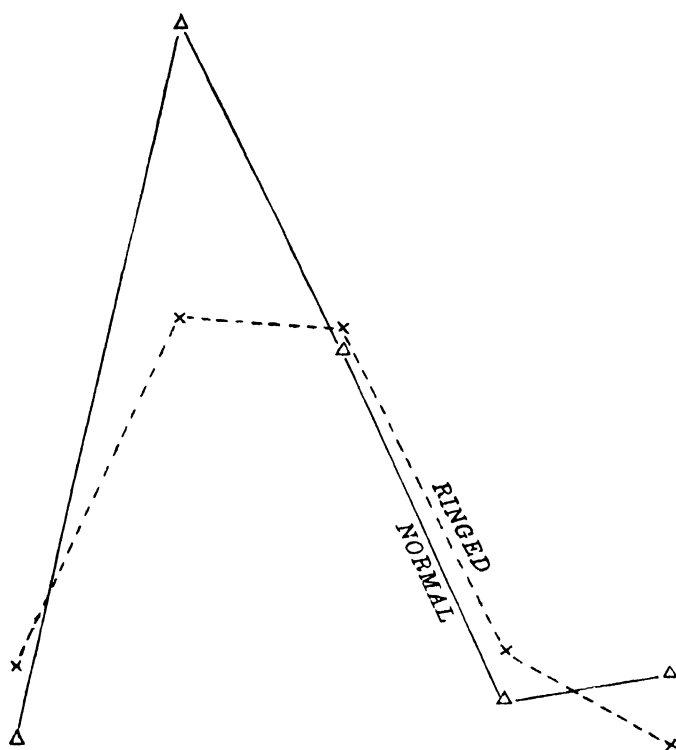
7 A. M.   11 A. M.   3 P. M.   7 P. M.   11 P. M.

FIG. 13. Concentration of reducing sugars in leaf of the ringed and normal (control) plants.

(1 divi. = 1 mgr.)

The rôle of reducing sugar in the jute plant does not seem to be very easy to comprehend. Thus it will be found that the quantity of reducing sugars in all the tissues analyzed was very small compared to that of sucrose, especially in wood where it was almost negligible. There was also a gradation

observed from leaf to bark and wood and a diurnal variation in each of the tissues (Fig. 3), but with all these it does not seem to be a translocatory sugar, for the fact that there was no accumulation of it in bark above a ring (Fig. 14).

In cotton plant it was found that there was an accumulation of sugar in bark, wood and leaf above a ring, and a depletion below that ring. From
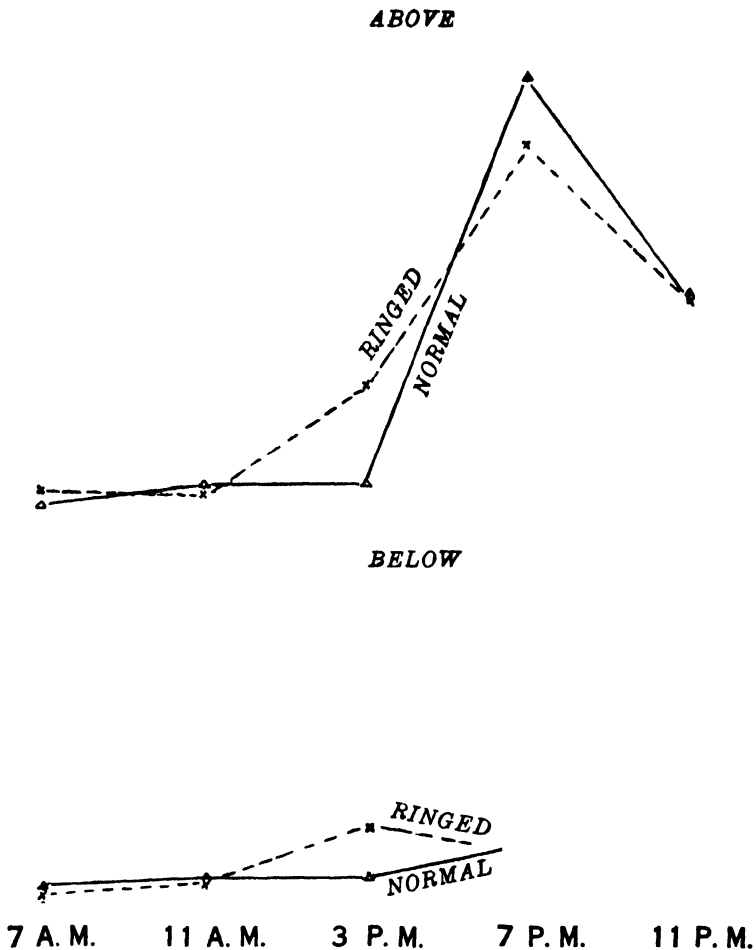
ABOVE

BELOW

7 A. M.   11 A. M.   3 P. M.   7 P. M.   11 P. M.

FIG. 14. Concentration of reducing sugars in bark of the ringed and normal (control) plants.

(1 divi. = 0·5 mgr.)

these observations it was contended that the ring brought about a stagnation of sugar in the tissues above it and by breaking the continuity of bark cut off the source of supply to the region below the ring. It was also thought that the

lateral translocation took place from bark to wood by diffusion for which sugar accumulated in wood above the region of the ring [10, 11]. In jute plant also there was an accumulation of sugar in bark above a ring and a depletion of it in bark below that ring (Fig. 8, table III). There was, however, no appreciable accumulation of sugar in leaf, which was perhaps too far off from the ringed
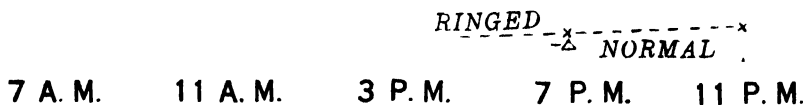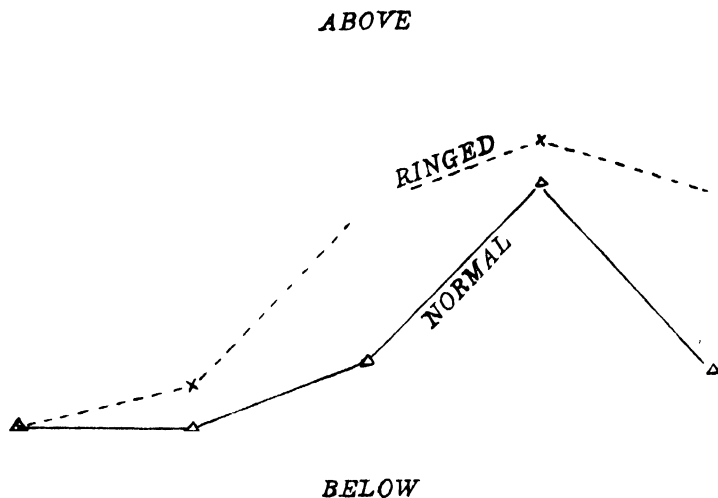
ABOVE

BELOW

RINGED ────ₓ─────────ₓ
           ────△ *NORMAL*

7 A. M.   11 A. M.   3 P. M.   7 P. M.   11 P. M.

Fig. 15. Concentration of reducing sugars in wood of the ringed and normal (control) plants.

(1 divi. = 0·1 mgr.)

region of the stem, but there was a tendency of an accumulation of starch in it (Fig. 7). The case of wood, however, was different. There was no accumulation of sugar in wood above a ring, although the adjacent bark was holding an extra quantity of this substance, and the depletion below a ring was not as marked as that of bark (Fig. 9). In fact the course followed by the depleting

sugar in wood of the lower region would suggest strongly that this part of the wood was getting a partial supply either from the wood above it or from the adjacent bark. But when lateral translocation seemed to be absent in the region above the ring, there was no reason why it should exist in the region below, and if subsequent work proves that there is no lateral translocation from bark to wood in jute plant, the results cited in this paper will strongly suggest that there is a parallel flow of translocatory sugar in the wood in smaller quantity compared to that in the bark. In this connection attention may also be drawn to the fact that the quantity of reducing sugars present in wood collected from regions both above and below a ring was found to be higher than that in the corresponding regions of the normal plant. Moreover, the reduced rate of sugar content in wood below a ring was due to the small quantity of sucrose found in that tissue (table III).

It may be of interest to mention some of the observations made in jute plants (not cited in the text) where single and double, full and partial rings were made in the stem. Within three or four days leaves began to droop down in plants where complete rings were made and in a week to ten days the plants began to dry up. In the stems the first sign of decay was observed in the intercepted regions between two complete rings, to be followed by the region below such a ring. Plants with single partial ring were, however, found to be normal, so also those where double partial rings were made in such a way that if both these rings were in the same plane they together could make a complete ring. This latter observation suggests that even if there be no lateral translocation from bark to wood, it may be a common phenomenon for the transference of translocatory substance from one part of the bark to the other [15].

A further discussion of these results will be made when more data are collected and presented later.

SUMMARY

Estimations were made of reducing sugars, sucrose and starch present in leaf, bark and wood of both normal and ringed jute plants at different periods of the day. Reducing sugars and sucrose were found in all the tissues analyzed, but starch was present only in leaf and was altogether absent in mature bark and wood.

Under normal conditions the quantities of reducing sugars, sucrose and starch present in leaf and bark showed an increase in the morning and a decrease towards the evening. The reducing sugars present in wood also underwent similar variations, but the quantities of sucrose present in this tissue continued to maintain an increasing rate. The highest level attained by the total sugars of leaf was earlier than that of bark. Maximum quantities of both reducing sugars and sucrose were found in leaf and the minimum in wood. Sucrose was present in very large quantities and underwent maximum variations.

In the ringed plants there was an accumulation of sucrose in bark above a ring and a depletion below it. From this and other considerations it is thought that sucrose is the sugar of translocation. In the wood above a ring there was no simultaneous increase of sucrose and this suggests that there may not be any lateral translocation of this sugar from bark to wood. There was also an accumulation of starch in leaf from the ringed plants. There existed a positive gradient of sugar from leaf to bark and from the upper part of the bark to the lower.

From various considerations it is suggested that although bark is the main channel of translocation of sugar, wood may also play a minor part in it, and there was some evidence that a parallel current with a very small quantity of sugar travels along the wood.

Local photosynthesis did not seem to contribute substantially to the quantity of sugars present in bark.

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REFERENCES

[1] Bennette, C. W.—1940: Bot. Review, Vol. 6, p. 443.
[2] Craft, A. S.—1931: Plant Physiol., Vol. 6, p. 1.
[3] ———— 1932: Plant Physiol., Vol. 7, p. 183.
[4] ———— 1933: Plant Physiol., Vol. 8, p. 81.
[5] ———— 1936: Plant Physiol., Vol. 11, p. 63.
[6] Curtis, O. F.—1935: 1st ed., McGraw-Hill Book Company, N.Y.
[7] Dixon, H. H.—1922: Nature, Vol. 110, p. 547.
[8] Engard, C. J.—1939: Bot. Gazette, Vol. 100, p. 439.
[9] Hanes, C. S.—1936: Biochem. Journ., Vol. 30, p. 168.
[10] Mason, T. G. and Maskell, E. J.—1928: Ann. Bot., Vol. 42, p. 189.
[11] ———— 1928: Ann. Bot., Vol. 42, p. 571.
[12] Mason, T. G., Maskell, E. J. and Phillis, E.—1936: Ann. Bot., Vol. 50, p. 23.
[13] Mason, T. G. and Phillis, E.—1936: Ann. Bot., Vol. 50, p. 455.
[14] ———— 1937: Bot. Review, Vol. 3, p. 47.
[15] Miller, E. C.—1938: 2nd ed., McGraw-Hill Book Company, N.Y.
[16] Münch, E.—1926: Ber. d. deutsch. bot. Ges., Vol. 44, p. 68.
[17] ———— 1927: Ber. d. deutsch. bot. Ges., Vol. 45, p. 340.
[18] ———— 1930: Jena, p. 234.
[19] Phillis, E. and Mason, T. G.—1933: Ann. Bot., Vol. 47, p. 585.
[20] Steward, F. C. and Priestley, J. H.—1932: Plant Physiol., Vol. 7, p. 165.
[21] Van der Plank, J. E.—1936: Biochem. Journ., Vol. 30, p. 457.
[22] Weevers, Th. and Westenberg, J.—1931: Konink. Akad. Wetensch Amsterdam Proc., Vol. 34, p. 1173.

XI. INVESTIGATIONS ON THE MINERAL CONTENT OF BLOOD OF BENGALI SUBJECT

1. TOTAL IRON, NON-HEMIN IRON AND COPPER CONTENT IN A FEW PATHOLOGICAL AND NORMAL CONDITIONS

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Our conceptions of the mineral nutrition of animals and the possible physiological significance of certain trace elements have been elucidated only a few years ago.

That copper is present in human blood has been recognized for nearly a century. Its concentration in the red blood corpuscle and serum of man and different animals of different ages has been estimated by several workers, though there is still a little controversy regarding the exact distribution of copper between the red blood corpuscles and serum. While Elvehjem and collaborators obtained higher values for red blood corpuscles, Schindel and others found more copper in plasma. Copper has been found to be present in the blood as an organic compound, chiefly linked with protein. Importance of copper as a supplement to iron for haemoglobin formation is at present admitted by all the workers (Hart *et al.*, 1927, 1928; Waddel *et al.*, 1929; Elvehjem and Sherman, 1932). Copper is not concerned with the assimilation of iron but functions in the conversion of absorbed iron into haemoglobin. It has no direct effect upon the formation of the erythrocytes of the blood except through its influence on haemoglobin formation. It has also been shown that copper feeding reduces the iron in the liver and tissues and increases the same in the blood (Cunningham, 1931).

The two classes of the biological iron compounds are the hemin iron and non-hemin iron compounds. Haemoglobin, cytochrome and other respiratory ferments belong to the former group. The latter group comprises those compounds whose chemical structure up to date must be considered as unknown. Quantitative determinations of this group are, however, very often performed by physiologists. The corpuscles have been found to contain much greater quantity of inorganic iron than the plasma. It is believed that in the new-born child a congenital reserve of non-hemin iron occurs and this compensates for the lack of iron in the milk diet during the first few months of suckling.

In view of the importance of the above findings, it appears that a knowledge of the variations of the copper and iron content of blood in various conditions would be of some value.

EXPERIMENTAL

Copper.—The method used was that of Callan and Henderson (1929) as modified by Eden and Green (1940). Blood was digested with a mixture of sulphuric acid and perchloric acid, followed by nitric acid. The digested colourless residue was diluted with water and sodium-pyrophosphate and liquor ammonia were added. Colour was developed by the addition of sodium-diethyl-di-thio-carbamate and the yellow complex that was formed with copper was extracted by shaking with amyl alcohol.

Non-hemin Iron.—This was extracted according to the method of Bruckman and Zondek (1940). To 1 c.c. of blood was added 5 c.c. of saturated sodium-pyrophosphate and 10 c.c. of 10% trichloracetic acid. The mixture was then heated in a boiling water bath for exactly 7 min., immediately centrifuged and the residue was washed twice with 4 c.c. of an equal mixture of the above two reagents. The united mixture which is ready for the determination of non-hemin iron forms a clear solution with a slight yellowish tinge. Colour was developed with thiocyanate after the addition of sulphuric acid to nullify the interference of pyrophosphate on the thiocyanate reaction.

Total Iron.—It was estimated according to the method of Kennedy (1927).

The colorimetric estimations were performed with Zeiss 'Stufen-photometer' using filters S 47 and S 50 for copper and iron respectively.

The result of estimation is given in table I. In all 12 samples of tubercular blood, 10 samples of new-born child, 5 samples each of diabetes and gastric ulcer, 15 samples of advanced pregnancy (7th–9th months); and 15 samples of normal, healthy and strongly built subjects of different ages have been studied.

TABLE I

Average of triplicate determinations
Result expressed in mg. per 100 c.c.

No.	Age	Sex	Condition of subjects	Total iron	Non-hemin iron	Copper
OPD 23/1	20	Male	Tuberculosis	38·5	2·3	0·25
OPD 26/1	15	,,	,,	41·0	3·2	0·31
4	22	,,	,,	43·0	2·8	0·30
4/388	40	,,	,,	30·0	1·7	0·24
95	23	,,	,,	28·0	2·3	0·25
436/7	15	,,	,,	40·5	1·5	0·18
92	40	,,	,,	36·0	1·8	0·19
39	18	,,	,,	41·0	1·2	0·33
10/3	24	,,	,,	42·0	1·6	0·15
38	21	,,	,,	33·0	2·3	0·28
27/3	22	,,	,,	27·0	2·7	0·28

TABLE I—*Contd.*

No.	Age	Sex	Condition of subjects	Total iron	Non-hemin iron	Copper
				Average of triplicate determinations Result expressed in mg. per 100 c.c.		
4/4	23	Male	Tuberculosis	45·0	1·5	0·13
108	..	,,	New-born child	37·0	1·9	0·26
XIII	..	,,	,,	39·0	1·2	0·21
104	.	,,	,,	27·0	2·1	0·33
30/1	..	Female	,,	37·0	1·7	0·22
12/3	.	,,	,,	43·0	1·5	0·22
28/2	..	,,	,,	41·0	1·3	0·13
100	..	,,	,,	37·0	1·6	0·20
12/3A		Male	,,	43·0	1·5	0·22
30/1A	..	,,	,,	38·0	1·5	0·27
OPD 22/1	31	Female	Pregnancy	32·0	1·6	0·20
OPD 27/2	27	,,	,,	28·0	0·8	0·10
OPD 28/2	25	,,	,,	35·0	1·0	0·15
28/2	19	,,	,,	30·0	1·0	0·14
26/3	41	,,	,,	33·5	1·3	0·16
104	35	,,	,,	38·0	1·2	0·15
10	35	,,	,,	36·0	0·6	0·15
147	29	,,	,,	32·0	1·8	0·18
5/3	21	,,	,,	32·0	1·0	0·18
31/3	28	,,	,,	38·6	1·5	0·14
108	32	,,	,,	34·0	1·0	0·18
A104	38	,,	,,	35·0	1·2	0·20
25/1	19	,,	,,	38·0	1·5	0·12
8/3	25	,,	,,	33·5	1·3	0·18
10/3	31	,,	,,	37·5	1·2	0·14
20/1	35	Male	Normal	39·0	1·0	0·12
22/1	38	,,	,,	41·0	1·2	0·12
27/1	52	,,	,,	40·0	1·5	0·16
28/1	19	,,	,,	32·0	1·5	0·18
4/2	21	,,	,,	38·0	0·85	0·14
4/58	21	,,	,,	41·0	1·2	0·14
20/2	27	,,	,,	40·0	1·6	0·14
20/31	31	,,	,,	46·0	2·0	0·10
21A	37	,,	,,	36·0	1·4	0·08
21/2	40	,,	,,	38·0	1 2	0·12
26	29	,,	,,	46·0	1·0	0·13
27	29	,,	,,	40·0	0·9	0·17
28	29	,,	,,	38·5	1·8	0·12
29	31	,,	,,	42·0	1·4	0·08
30	39	,,	,,	46·0	1·6	0·15
2	35	,,	Gastric Ulcer	32·0	0·8	0·16
56	36	,,	,,	40·0	1·2	0·22
98	40	,,	,,	40·0	1·5	0·20
95	28	,,	,,	36·0	1·2	0·20
28/3	43	,,	,,	37·0	1·2	0·20
375/1	46	,,	Diabetes	27·5	0·75	0·22
1609/1	55	,,	,,	31·0	1·1	0·18
87	65	,,	,,	30·5	1·7	0·20
149	59	,,	,,	43·0	1·0	0·10
102	70	Female	,,	34·0	1·3	0·18

TABLE II

Average of total iron, non-hemin iron, hemin iron and copper in the various conditions
studied

Condition of subject	mg. per 100 c.c.				Ratio	
	Total iron	Non-hemin iron	Hemin iron	Copper	$\dfrac{\text{T.F.}}{\text{N.H.F.}}$	$\dfrac{\text{T.F.}}{\text{Cu}}$
Normal	40·1	1·3	38·8	0·13	30	308
Tuberculosis	37·1	2·1	35·0	0·24	18	154
New-born child	37·2	1·6	35·6	0·22	23	165
Diabetes	33·2	1·2	32·0	0·18	28	184
Gastric Ulcer	37·0	1·2	35·8	0·19	31	190
Pregnancy	34·2	1·2	33·0	0·16	29·5	214

T.F. = Total iron.
N.H.F. = Non-hemin iron.
Cu = Copper.

TABLE III

The total iron, non-hemin iron and copper content as determined by different authors
is shown below

Author	Type of blood	Result expressed in mg. per 100 c.c.		
		Total iron	Non-hemin iron	Copper
Tompsett (1934)	Human whole blood	..	1·19	0·185–0·229
Macfarlane (1932)	,,	51 5	..	0·185–0·210
Schonheimer (1929)	,,	.	.	0·113–0·114
Barkan (1928)	,,	..	1·7	..
Kennedy (1927)	,,	51·0	..	..
Locke (1932)	Human serum	.	..	0·008–0·095
Warburg (1927)	,,	..		0·124
Barkan (1927)	,,	.	0·084–0·075	
Guillemet (1931)	Plasma	.		0·056–0·075

DISCUSSION

The haemoglobin content of normal blood in European countries is
14·5–17 gm. per cent. Assuming that 1 gm. haemoglobin contains approxi-
mately 0·0032 gm. iron the haemoglobin iron content would be about 48–55 mg.
per cent. Normal Indian blood is believed to contain 12·5–14 gm. per
cent of haemoglobin and the corresponding iron value would be 40–45 mg.
per cent. Hemin iron determined by me is 39 mg. per cent in normal Bengali
subjects. A marked reduction in the total iron content occurs during the
later part of pregnancy. It is well known that a disturbance in the general

metabolism takes place in the diabetic patients; total iron content appears to sink considerably below normal in the diabetic patients (Fig. 1).

FIG. 1
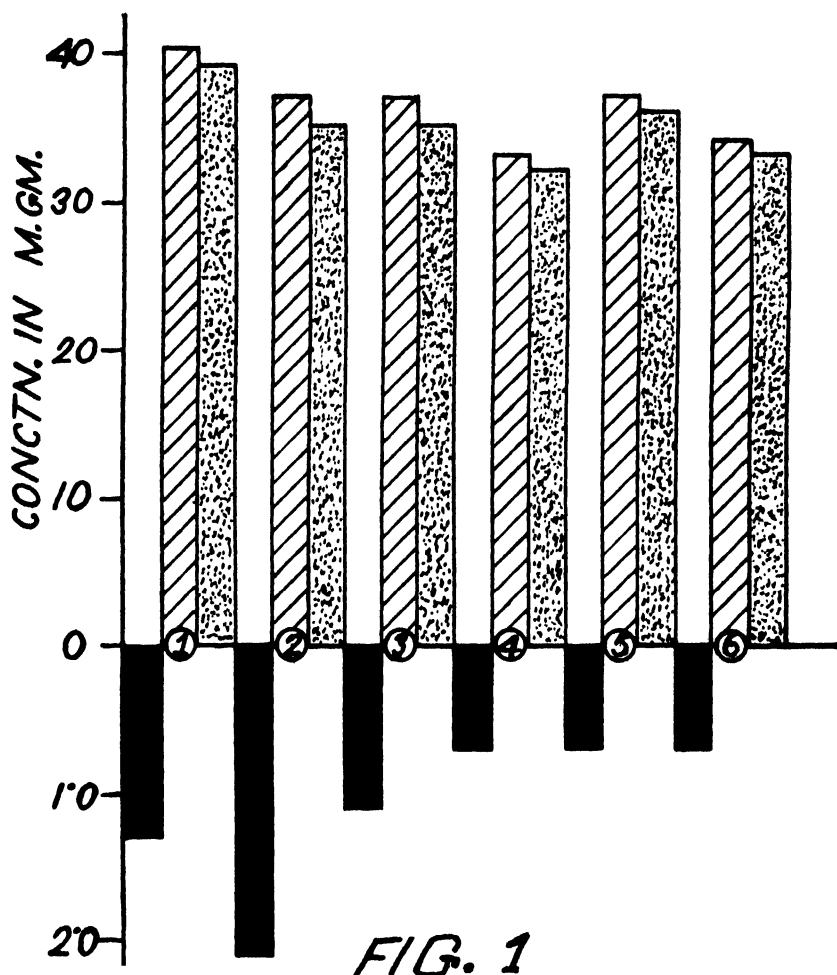
1. Normal.   2. Tuberculosis.   3. New-born child.
4. Diabetes.   5. Gastric Ulcer.   6. Pregnancy.

Deep shaded ..   Non-hemin iron.
Striped   ..   Total iron.
Dotted   ..   Hemin iron.

The non-hemin iron content in normal subjects is 1·34 and may be considered practically of the same order as found by Tompsett. It may be pointed out that the non-hemin iron content does not seem to decrease sufficiently below normal in the different pathological condition studied to warrant a significant finding save and except in tuberculosis and in the new-born child.

In tubercular patients the value is about 60% above normal, whereas in the new-born child it is 25% (Fig. 1).

The copper content of normal subjects has been found to be 0·13 mg. per 100 c.c. of whole blood, a value which may be considered a little below than the value found by Tompsett and Macfarlane.· Whether this discrepancy is due to the different technique employed or it is due to a natural decreased content in the Bengali blood requires reinvestigation. The tubercular patient and the new-born child contain significantly higher quantity of copper in their blood (Fig. 2). It has been observed by other authors too that a definitely
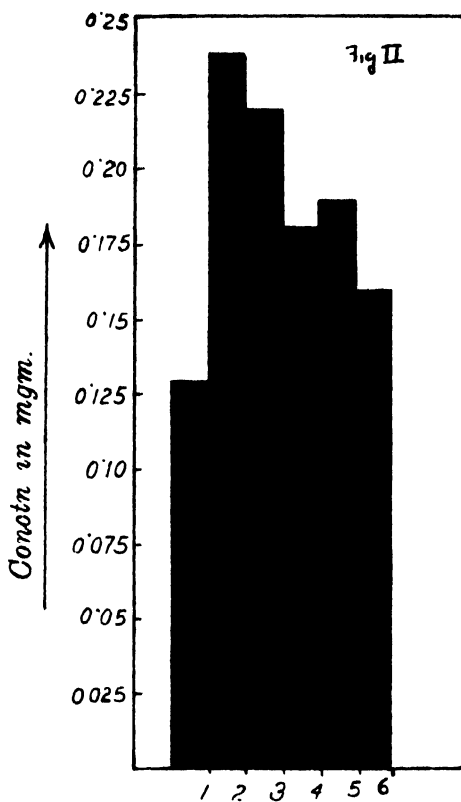
FIG. 2. (Copper.)

1. Normal.
3. New-born child.
5. Gastric Ulcer.

2. Tuberculosis.
4. Diabetes.
6. Pregnancy.

increased value of copper takes place in tuberculosis, infections and in ill-nourished subjects (Bruckman, 1939; Cherbuliez, 1929; Ramage, 1933; Heilmeyer, 1938; Eggleton, 1940). The significance of this phenomenon is at present not known. Numerous investigators have, however, pointed out that

the concentration and absolute amount of copper in the mammals are higher at birth than at any other time during life.   Ramage (1938) has shown that the human liver contains the maximum amount of copper at birth than at any other time during intrauterine life.   The percentage of copper at term was found by him to be nearly double than at 24 weeks' development.   It is believed that this mobilization of copper in the foetal liver is a protective measure for the successful continuance of normal haemopoiesis during the suckling period.   As will be noticed from Fig. II, a general rise in the copper content occurs in all the pathological cases studied.

SUMMARY

The total iron, non-hemin iron and copper content of blood of Bengali subjects have been determined in normal and a few pathological cases.   A significant increase in the non-hemin iron and copper content occurs in tuberculosis and in the new-born child.   The total iron content sinks considerably below normal in diabetes and during pregnancy.

REFERENCES

[1] Hart, Elevehjem, Waddel and Herin (1927), J. Biol. Chem., 72, 299.
[2] Hart, Elevehjem and Steenbock (1928), J. Biol. Chem., 77, 297.
[3] Waddel, Steenbock and Hart (1929), J. Biol. Chem., 83, 243.
[4] Elvehjem and Sherman (1932), J. Biol. Chem., 98, 309.
[5] Cunningham (1931), Biochem. J., 25, 1267.
[6] Callan and Henderson (1929), Analyst., 54, 650.
[7] Eden and Green (1940), Biochem. J., 34, 1202.
[8] Bruckman and Zondek (1939), Biochem. J., 33, 1845.
[9] Kennedy (1927), J. Biol. Chem., 74, 385.
[10] Bruckman and Zondek (1940), J. Biol. Chem., 135, 23.
[11] Cherbuliez (1929), C.R. Soc. Phys. Geneve, 46, 144.
[12] Ramage and Sheldon (1933), Pro. Roy. Soc. B., 113, 308.
[13] Heilmeyer (1938), Klin. Wochschr., 17, 213.
[14] Eggleton (1940), Biochem. J., 34, 991.
[15] Tompsett (1934), Biochem. J., 28, 1544.
[16] Macfarlane (1932), Biochem. J., 26, 1022.
[17] Schonheimer (1929), Z. Physiol. Chem., 180, 249.
[18] Locke (1932), J. Clin. Invest., 11, 527.
[19] Warburg (1927), Biochem. Z., 187, 255.
[20] Guillemet (1931), Compt. Rend. Soc. Biol., 108, 32.
[21] Barkan (1927), 2 Physiol. Chem., 171, 194.

XII. STUDIES IN THE PHYSIOLOGY OF SOME INDIAN FRUITS

II. Catalase and Oxidase Activity in Mangifera indica

By Hirendra Kumar Banerjee, M.Sc. *and* Baikuntha Kumar Kar, M.Sc., Ph.D.

Introduction

In recent years the importance of catalase in the oxidation of biological substrates has become more prominent due to the investigations of Zeile and Hellstrom [1], of Sumner and Dounce [2], of Agner [3], of Keilen and Hartree [4] and of Stern [5] who have demonstrated that catalase is a chromoprotein—a combination of prosthetic group containing haematin compound with a protein analogous to haemoglobin. The catalytic activity of catalase is accompanied by a change in valence of its Fe, i.e. the catalytic decomposition of hydrogen-peroxide is brought about by successive reduction of the catalase ferric iron to ferrous iron and its reoxidation by the oxygen formed.

Catalase is universally found in vegetative cells and a large amount of work has been done on its rôle in respiration, in vitality of seeds and plant organs and its function in various physiological activities. The catalytic action of catalase in case of fruit growth and development is not yet clearly understood as is evident from some of the literature considered below.

Neller [6] found a higher catalase content in Jonathon apples passing through the breakdown process but a lower activity was noted in the advanced stages of breakdown. In normal fruits, the catalase activity increased during early storage and decreased late in storage life. Catalase can, therefore, according to Neller be taken as an index of metabolic activity. Drain [7] could not find a close correlation between respiration rate and catalase activity in apple varieties. De Villiers [8] working with grapes found an increased catalase activity with increasing maturity of the fruit but there was a slight decrease when the grapes became fully ripe. Oxidase activity and respiration rate decreased, however, with maturity. Gustafson [9] and his collaborators have found a close correlation with respiration rate, growth and catalase activity in tomato fruits. Harding [10] worked with different temperatures and found no correlation between catalase activity and respiration at a lower temperature of 30° and 36°F. but at 50°F. there was a correlation in case of Grimmes Golden apples. Magness and Ballard [11] found that the highest point in catalase activity was found prior to the period of greatest respiratory activity in Bartlett pears stored at 60°. Ezell and Gerhardt [12] in their investigations on apple and

pear fruits have concluded that oxidase and catalase activity were not directly correlated with the rate of respiration or with each other, in fruit subjected to various storage temperatures or to various chemical respiratory stimulants or depressants. There was, however, a positive correlation between the rate of respiration, oxidase and catalase activity in Bartlett pear fruits on the tree from earliest stage to maturity. The inter correlation of respiration, catalase and oxidase failed to hold in fruits removed from the tree and kept in storage.

It is therefore seen that conflicting results have been obtained by different workers on the catalytic reaction of the fruit substrates. The catalytic action of catalase is dependent upon its prosthetic group containing haemin Fe, and an investigation on this aspect may provide some illuminating results on the substrate oxidation. In the following investigation an attempt has therefore been made to study the catalase activity throughout the life-cycle of the mango fruit and its relation to its Fe content and substrate concentration. The behaviour of catalase activity has also been studied by indirectly changing the substrate concentration through different storage conditions and ethylene treatment.

Material.—The material for the investigation was collected from a mango tree of Alphonso variety growing in the Institute gardens. The fruits were numbered at the very early stage of fruit-setting so as to keep a record of the age of the fruits at the time of collection. Each time 12 fruits of the same age were collected from all parts of the tree in order to avoid variations and throughout the period of investigation the experimental material was taken ·from the same tree. The first picking was made when the fruits attained the age of 13 days and the subsequent pickings were made from time to time, during the whole season of about four months. The last estimation was made when the fruits were 120 days old. The fruits were collected in the morning and the experimental treatments began almost immediately. The storage experiments were also conducted simultaneously at different stages. For the estimation of Fe the fruiting shoots were removed and the leaves were separated. Fe was estimated separately in fruits, leaves and shoot portion.

Method.—The material was prepared for the following estimations— catalase, peroxidase, total iron, non-haemin iron and haemin iron.

Catalase.—For the estimation of the catalase activity recourse was taken to the volumetric study of decomposition of mono-ethyl hydrogen peroxide by the Iodometric method of Stern [18]. From each of 12 mangoes one gm. of carefully weighed pulp was taken and thoroughly macerated with quartz sand in the presence of 5 c.c. of M/20 phosphate buffer pH 6·7 and the whole transferred into stoppered flasks. The bottles were kept in an incubator set at 37°C. for 10 minutes to raise the temperature of the contents to that of the incubator. Stern carried out his experiments at the laboratory temperature. As the local temperature varied from 25°C. to 35°C. during the mango season we found it convenient to work throughout at 37°C.

The experiment was started by adding to each of the flasks 10 c.c. of 1·1 M mono-ethyl hydrogen peroxide prepared according to the method of Baeyer

and Villiger[14] as modified by Rieche and Hitz[15] and the bottles vigorously agitated. After exactly five minutes the reaction was stopped by the addition of 10 c.c. of 33% H_2SO_4, 5 c.c. of 10% potassium iodide solution and 3 drops of molybdic acid solution were then added to each bottle. After standing for exactly one hour the liberated iodine was titrated with 0·1 N. thiosulphate. The catalase activity is expressed by the milligram of oxygen liberated per gm. of the mango pulp (fresh weight). A blank experiment with boiled enzymatic extract with all the reagents employed was run alongside. This (blank) reading minus the experimental titre gives the equivalent of peroxide decomposed by the mango catalase. The average of twelve such determinations was taken for each experiment.

Oxidase.—For this purpose the Iodometric method adopted by Guthrie[16] for the determination of oxidase activity in potato juice was followed. Alkaline glucose prepared as detailed below was used as substrate for oxidation.

Preparation of the substrate : 40 gm. of glucose is dissolved in 400 c.c. of N. NaOH in a pyrex bottle and kept immersed in a water bath at 80°C. for 15 minutes. The bottle is removed and the contents neutralized immediately by adding 80% phosphoric acid. To the mixture is added 20 gm. of decolourizing charcoal and the whole left aside for 18 hrs. The mixture is filtered and the filtrate again treated with 20 gm. of norit charcoal and filtered. The pH of this solution is adjusted to 6·5 by addition of a few drops of N. NaOH or N. HCl as necessary. This is kept in a stoppered bottle in a refrigerator in the dark and it is found to keep well for weeks. This stock solution is diluted with an equal volume of water before use.

The method of estimation : 25 c.c. of the above substrate solution is taken in each of a dozen of aeration tubes. To each of these is added the enzyme preparation exactly as in the case of the catalase experiment. The only difference being that the buffer used was at pH 6·5. Five drops of paraffin oil were added to each tube which was then aerated for 1 hr., after which the mixtures were washed into stoppered bottles containing 25 c.c. of 10% trichloroacetic acid adding in all 50 c.c. water. 50 c.c. of N/50 Iodine solution is added to each and the bottles left aside for 30 minutes. Excess iodine is then titrated against N/10 Hypo. A blank with boiled enzymic extract is run alongside and titrated first. The difference is a measure of the oxidase activity of the sample. The average of these twelve determinations is taken as a measure of the activity of the sample. The oxidase activity is expressed as mg. glucose oxidised per gm. of fresh pulp.

Total Iron.—Cut pieces of the mango pulp (from 12 mangoes mixed together) without the skin and the stone were crushed in a pestle and mortar and 1 gm. carefully weighed out in a Kjeldahl flask. The material was digested in 5 c.c. H_2SO_4 and perchloric acid mixture. The clear digest was made up to 50 c.c. A small quantity of dil. HNO_3 was also added. The Fe content was then estimated according to Kennedy by the colorimetric method with KCNS solution using the Pulfrich photometer with the light filter S 50. From the

13

extinction coefficient readings the quantity of Fe was obtained by reference to a calibration curve drawn previously of Ferric alum. Duplicates were run throughout.

Non-Haemin Iron.—For this purpose the method adopted by Bruckmann and Zondek [17] was utilized. 1 gm. of tissue from the same sample mentioned above was ground in a mortar with powdered glass, 5 c.c. saturated Na-pyrophosphate and 10 c.c. of 10% trichloroacetic acid. The mixture was quantitatively transferred into centrifuge tubes and both heated in boiling water for exactly 7 minutes, immediately centrifuged and the residue washed repeatedly with a mixture of the two reagents and made up to volume, and aliquotes were wet ashed by H_2SO_4 and $HClO_4$. Subsequent determination with the extinction photometer was made exactly as in the previous case.

Haemin Iron.—Direct determination of Haemin iron was performed by the method of Yabusoe [18]. 1 gm. tissue pulp ground with powdered glass, 1 c.c. N. HCl and 5 c.c. ice cold absolute methanol, the mixture transferred to a centrifuge tube and centrifuged. The supernatant liquid was poured out and the sediment washed with methanol until the washings were colourless. The collected filtrates were shaken with 1 gm. powdered $MgSO_4$ (iron free) and after 30 minutes centrifuged. The clear solution is made to volume and aliquotes ashed with H_2SO_4 and perchloric acid and the iron determinations carried out as before.

EXPERIMENTAL RESULTS

Catalase activity.—The first sample for analysis was collected when the fruits were 13 days of age and the average fresh weight of the fruit was only one gram. The growth and development of the fruit as indicated by the increase in fresh weight showed rapid increase during the early stages up to 3 months after which there was little increase in fresh weight. Within the period of 120 days it was seen that the fruits increased by about 160 times the fresh weight.

Tracing the catalase activity from the earliest stage of fruit-setting, it was found that in the earliest stage here estimated (1 gm. fresh weight) the catalase activity was very low being 0·12. As the fruit gradually increased in size and weight, the catalase also showed a very slow and steady increase to 0·20, i.e. double the amount from the earliest stage within a period of about 44 days of active growth and development. From now on the fruit showed a very rapid increase in its fresh weight and the catalase also showed increased activity, attaining four times its former activity. From the age of 57 days and onwards up to 80 days in age, there was a rapid increase in catalase activity. The next period from 80 days to 99 days, i.e. about 19 days the growth of activity curve was more or less flattened and the increase was not so marked. But from this point further, the activity was very rapid reaching the maximum limit at near about 115 days in age of the fruit and then found steeply to decline. In Table I

the age of the fruit, its average fresh weight, its catalase and oxidase activity with haemin iron content have been summarized. Some simultaneous estimation made of the vitamin C content have also been given.

TABLE I

Age in days from fruit-setting	Average fresh weight in grams	Catalase mg. O_2-per gm. pulp	Oxidase mg. glucose oxidized per gm. pulp	Haemin Fe mg. per gm. pulp	Vitamin C mg. per gm. pulp
13	1	0·12	0·07	0·016	..
42	5	0·15	0·08	0·012	2·6
57	15	0·20	0·11	0·036	..
72	60	0·88	0·12	0·125	..
82	65	2·00	0·20	0·149	1·7
84	99	3·75	0·25	..	..
99*	150	4·20	0·30	0·158	..
106	150	13·5	0·35	0·151	0·86
112	..	52·0	0·65	..	..
114	150	64·7	0·80	0·172	..
115	..	78·4	0·85	0·178	..
117	..	65·0	0·45	..	..
120	..	48·0	0·40	0·172	..

* At this stage ripening begins.

The behaviour of the catalase therefore showed a steady and continuous increase with the progressive growth and development of the fruit on the tree. But this increase in catalase was seen to be marked by definite periods of activity. It was found previously that according to the biochemical nature and the behaviour of the substrate concentration, the fruit in its life-cycle showed definite physiological stages (Kar and Banerjee [19, 20]) and the trend of the catalase curve seems to fit well within these stages (Fig. 1). It will be seen from the curve that during the early and the enlarging stages there was a gradual and steady increase in the catalase activity. In mango fruit the hardening of endocarp (stone-formation) is of a great physiological importance, influencing important changes in the chemical substrate concentration. With the beginning of endocarp hardening a causal increased activity in the catalase was also noticed. The effect of the hardening in the fruit substrate is not known, but the increase in catalase no doubt indicates a change to vigorous metabolic activity. The stage of the fruit from 80 days to 100 days of growth and development showed a phase of higher level catalase activity, which corresponded to the region of the mature stage of the fruit, as after this stage, the fruits showed signs of ripening. With the beginning of ripening the catalase further showed an enhanced rate of activity (Bagster [21]) reaching a maximum and then declining, when definite physiological breakdown sets in in the post-ripening stage. Even in over-ripened and deteriorated condition of the fruit a certain amount of catalase activity was found. If catalase activity is to be taken as a measure of

oxidation rate in the fruit substrate, then higher catalase efficiency was noticed to begin in the mature stage of the fruit where the highest respiratory efficiency, i.e. the climacteric is supposed to lie.
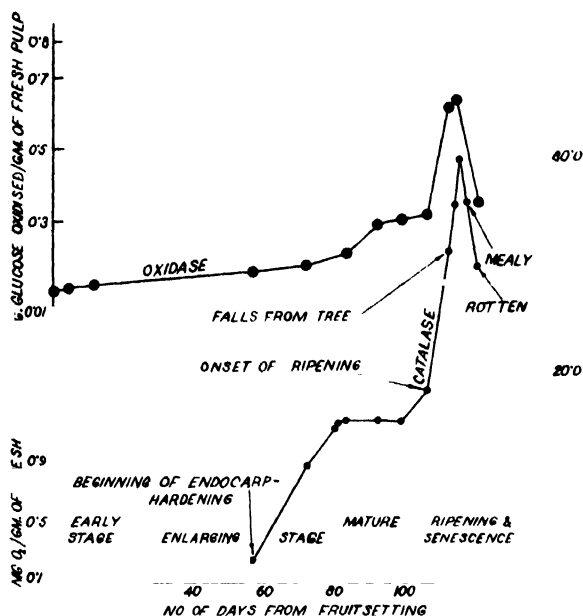

FIG. 1.   Showing catalase and oxidase activity during the life-cycle of the fruit on the tree.

Oxidase activity.—The different phases of activity as shown by catalase, could not be detected in case of oxidase. From the earliest stages up to the onsetting of ripening a gradual and steady increase in oxidase activity was noticed. There was no steep rise in the enlarging stage, in the region of endocarp hardening as was the case in catalase. The only steep rise was noticeable with the onsetting of the ripening stage, which was comparable to the behaviour of catalase in the same region. Oxidase like catalase attained the maximum activity in the same stage of ripening and then declined rapidly. All along the life of the fruit, though oxidase gradually showed increasing activity till the ripening stage yet from the trend of the curve, the different physiological stages of the fruit, could not be demarked. The oxidase activity and the catalytic activity of the catalase, therefore, attained vigorous activity in the later stages of fruit growth and development, when also the various substrate concentrations were subject to more active metabolic changes.

Variations in Fe content.—The prosthetic group of catalase containing haemin Fe was estimated simultaneously in the fruit substrate throughout the period of investigation. The total iron was estimated in order to find the distribution of Fe separately in fruits, leaves and shoot portions during the complete life-cycle of the fruit.

The variations in the haemin Fe content of the fruit throughout its life-cycle showed a relationship with the catalase activity and the trend of the changes, over a greater period, showed similar variations according to the different stages of the fruit.   In Fig. 2 the variations in haemin and total Fe
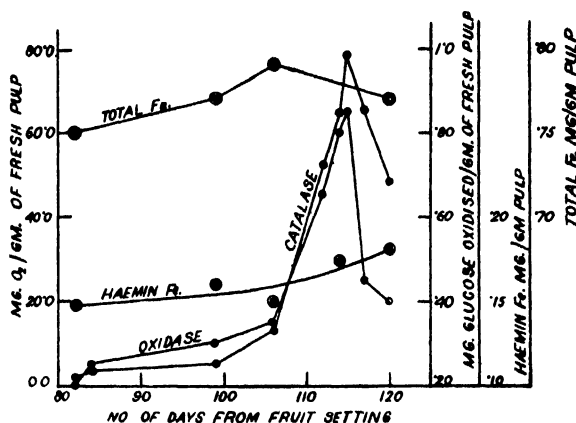

FIG. 2.   Showing the variations in haemin and total Fe content together with the catalase and oxidase activity in the later stages of the fruit.

content together with the catalase and oxidase activity in the later stages of the fruit from 80 days onwards have been given.   In the ripening stage of the fruit when catalase showed an enhanced activity, reaching a maximum, the haemin Fe also increased; but later with the decline of the catalase, haemin Fe did not show any decline but remained more or less constant.

The total iron content also showed a progressive increase with the growth and development of the fruit.   The variations in increase were found to be more in early stages than in the later stages.   In Table II the distribution of total Fe in different parts of the shoot (leaf, stem and fruit) have been given.

TABLE II

Showing distribution of total Fe in mg./100 gm. fresh pulp

Age in days from fruit-setting	Leaf A	Stem (shoot portion) B	Fruit C	A+B+C	Prop. of haemin Fe to total Fe
13	62·3	50·1	10·8	123·2	6·7
42	85·2	52·6	44·2	182·0	21·0
57	83·0	52·8	58·1	193·9	16·1
72	79·2	52·5	68·8	200·5	5·5
82	77·6	52·0	74·5	204·1	5·0
99	74·1	50·1	77·0	201·2	4·8
106	73·1	49·8	78·9	201·8	5·2
120	69·8	49·1	77·2	196·1	4·0

In the ripening stage the total Fe content showed rather a decrease.   The proportion of haemin Fe to total iron in the fruit showed fluctuation in the early stages but later the proportion remained constant and within a certain range.   In fruit therefore a correlation between the catalase and haemin and total Fe content could be seen.   This correlation was, however, not seen in the post-ripening stage of the fruit.

The distribution of total Fe in stem (shoot portion) and leaves at the successive stages of the fruit growth and development showed a greater fluctuation in the leaves than in the shoot portion.   In the early stages of the fruit growth of about 42 days to 57 days, the leaves showed a greater Fe content than in the later stages when it was considerably depleted (Fig. 3).   The variation
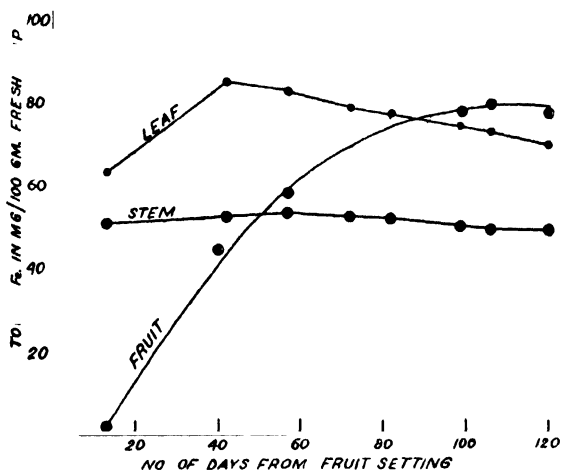
Fig. 3.   Showing the distribution of total Fe in flowering
shoot of Mangifera indica.

of total Fe in the leaves was primarily due firstly to the age of the leaves and secondly to the possible translocation to other plant organs of which the fruit is one.   In the stem (shoot portion) a steady concentration of iron content was maintained throughout the season.   When the total Fe content in the leaves gradually decreased the corresponding total Fe content in the fruit increased and in later stages fruit had more total Fe content than either the leaves or shoot portion.   The increase of the total Fe content in the fruit was therefore compensated by a depletion in the iron content of the leaves, which together with the elaborated food material in them, act as the source of food supply to the fruits.   There was therefore a steady migration of Fe from the leaves through the stem to the fruit and this may be taken as the source of haemin Fe for the prosthetic group of catalase.

If the total Fe content of the leaves, shoot portion and fruits were considered together then an interesting variation in the form of a depletion was noted in the later stages from the time when the fruits were about 82 days in age.   It is

possible to explain this loss of Fe by considering the fact of translocation of Fe to the developing stone and embryo at this stage of the fruit.

Catalase activity under different conditions of storage.—It has been seen before that catalase activity was greatly enhanced during the later stages of the fruit. A comparatively greater activity was noticed when the fruit was passing from the mature stage to the ripening stage. The fruits at this transition stage were subjected to different kinds of storage. One set was treated with ethylene at a temperature 28°–32°C., the other set was kept in cold storage in a refrigerator at a temperature 8°–12°C. and the third set was left under room conditions (28°–32°C.). This room temperature set acted as control for the other two sets. The variations in catalase activity under different storage conditions have been represented in Fig. 4. It was seen that
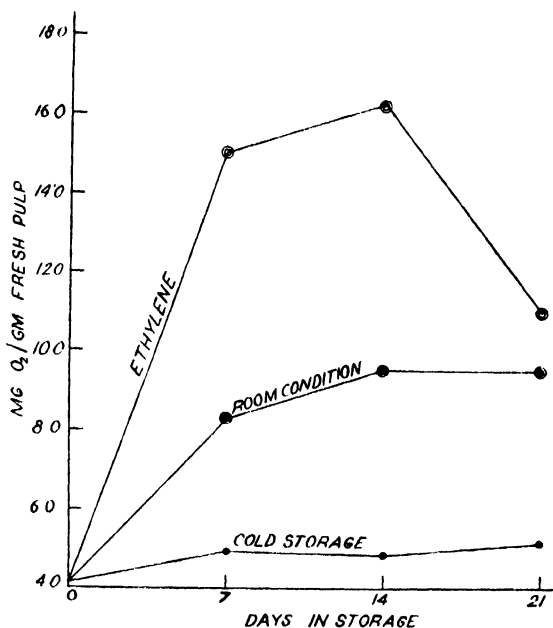
FIG. 4.   Showing catalase activity in mature mango fruits under different conditions of storage and ethylene treatment.

catalase activity was much slowed down in cold storage. It was known from previous investigation that physiological breakdown was slowed down during storage under low temperatures and the slow catalase activity was due to the reduced metabolic activity under cold storage. It was therefore expected that catalase which was associated with the oxidation process would also diminish with reduced respiration rate at lower temperatures.

The fruits which were on the other hand left under room conditions under high temperature showed increased catalase activity during the period of

storage. The activity was increased to more than twice the amount found in the beginning of the storage.

Ethylene was found to have a marked effect on the catalase activity which increased to four times the original value, after a period of fourteen days storage and when kept further in storage it showed a lower value. The increased catalase value under ethylene treatment may be correlated with the increased rate of respiration, with the strong hydrolysis of starch and the increased output of sugars in the substrate concentration which were also found, as some of the similar effects, as a result of ethylene treatment.

Discussion.—From the description of the foregoing results on the enzymatic activity in the substrate of the mango fruit at different stages of its growth and development, it was interesting to note that catalase and oxidase showed in their activities definite periodic variations which could be safely correlated with the different physiological and biochemical stages of the fruit. From fruit setting to the fully ripened stage, the catalase activity could be well marked into five distinct phases, viz.: (1) an early phase of very low activity, (2) a phase of rapid and steady increase, (3) a period of higher level activity with, (4) steep rise to a maximum activity, and lastly (5) a rapid decline to a minimum value. All these stages passed one after the other in the life-cycle of the fruit.

When the different phases were further analyzed in relation to the available chemical substrates and the metabolic drifts in the life-cycle of the fruit, it became apparent that the first stage of very low catalase activity was associated with the protein nature of the substrate in this stage of the fruit. As the fruit developed with the appearance of intercellular spaces and the appearance of chemical substrates in the form of starch and sugars the catalase activity also became markedly enhanced. The period of higher level activity which was shown for a period of 20 days beginning from the age of 80 days, corresponded with the mature stage of the fruit.   At this stage the highest respiratory efficiency of the fruit was also expected.   Therefore the enhanced rate of catalase activity was correlated with the enhanced metabolic changes in the substrate concentration.

A positive correlation with enhanced catalase activity and high respiratory efficiency of the fruit was seen in the mature stage of the fruit, i.e. so long the fruit was attached to the tree. But from estimation made with the fruits, after they have dropped down from the tree, a maximum catalase activity was noted. After the maximum the declining tendency was seen when the fruits have attained the full ripening stage and possibly on way to physiological breakdown. Therefore if the whole life-cycle of the fruit was considered the oxidative enzymes (catalase and oxidase) reached the maximum activity rather in the post-climacteric, i.e. with the onsetting of the ripening stage, bearing the fact in mind that this maximum activity was seen in detached fruits, after they have completed their normal life-cycle on the tree.

The catalase activity therefore may be regarded as an index of metabolic activity and was closely correlated with the different phases and respiratory

efficiency of the fruit.   The above holds good with fruits growing normally on tree.   But with detached fruits the correlation was found not to be tenable as was also noted by Ezell and Gerhardt [12].

The results obtained on catalase activity by influencing the chemical substrates of the fruit through different kinds of storage showed that cold storage which depressed the respiration rate and the metabolic activity also depressed the catalase activity.   On the other hand, ethylene which was found to act as a stimulant to respiration rate (Kar and Banerjee [19]) also stimulated the catalase activity.   These observations of course were confined only with fruits of the mature stage but clearly corroborated the fact as stated above that catalase activity is an index of metabolic changes.

From the end products it is very difficult to know the nature of the catalytic action of catalase.   We were not able to derive any help in this respect from our analysis of the prosthetic group of catalase containing Fe.   During the life-cycle of the fruit, the total Fe and haemin Fe showed a gradual increase and there could be seen a progressive correlation with the catalase activity up to a certain stage of the fruit.   But in the post ripening stage (in detached fruits) when catalase decreased the Fe contents did not show any decrease.

This may be explained on the assumption that with the beginning of the physiological breakdown in the post-ripening stage, the protein nature of the catalase was also affected, hence the decrease in catalase activity; but the content of the mineral constituent Fe remained intact.

From the above discussion it became evident that catalase and oxidase as respiratory enzymes were closely functioning in the oxidation-reduction processes in the fruit substrates.   The physiological development of the fruit consisted in a series of biochemical changes in the substrates and the close variation of catalase and oxidase with these changes indicated the enzymic nature of these reactions.

SUMMARY

(1) The catalase activity in mango fruit from fruit-setting to ripening stage, while growing normally on tree, showed distinct phases of activity which were correlated with the different physiological and biochemical stages in the fruit growth and development.

(2) The different phases could be distinguished into (a) an early phase of very low activity, (b) a phase of rapid and steady increase, (c) a period of higher level activity, (d) a steep rise to maximum activity, and (e) rapid decline.

(3) Throughout the life-cycle of the fruit on tree a close correlation existed between the catalase activity, the enhanced metabolic activity and the respiratory efficiency of the fruit.   This correlation was, however, not apparent when the fruits were detached from the tree.

(4) Oxidase showed a progressive increase in its activity with the advancing growth and development of the fruit.

(5) A positive correlation was shown between the catalase and oxidase activity of the fruit with the haemin Fe content of the tissue at that stage.

(6) Fruits of mature stage showed increased catalase activity under ethylene treatment than under cold storage or room condition.

(7) The distribution of total Fe and haemin Fe showed a progressive accumulation of Fe in the fruit with its onward march towards maturity.

(8) The vitamin C content of the fruit decreased in the later stages, showing maximum in the early stages.

LITERATURE

[1] Zeile, K. and Hellstrom, H., (1930).—Z. Physiol. Chemie., 192, 171.

[2] Sumner, J. B. and Dounce, A. L., (1937).—Jourl. Biol. Chem., 121, 417.

[3] Agner, K., (1938).—Biochem. J., 32, 1702.

[4] Keilen D. and Hartree, E. F., (1937).—Proc. Roy. Soc., B, 124, 397.

[5] Stern, K. G., (1937a).—J. Gen. Physiol., 20, 631.

———— (1937c).—J. Biol. Chem., 121, 561.

[6] Neller, J. R., (1931).—Plant Physiol., 6, 347.

[7] Drain, B. D., (1926) —Bot. Gaz., 82, 183.

[8] De Villiers, F. J., (1926).—Union S. Africa Dept. Agr. Sc. Bull., 45, 97.

[9] Gustafson, F. G., Clark, I., Shaw, D. A. and Warweg, E., (1932).—Plant Physiol., 7, 155.

[10] Harding, P. L., (1935).—Iowa Agr. Expt. St. Res. Bull., 182, 317.

[11] Magness, J. R. and Ballard, W. S., (1926).—Journ. Agr. Res., 32, 801.

[12] Ezell, B. D. and Gerhardt, F., (1938).—Jour. Agr. Res., 56, 365.

[13] Stern, K. G., (1936).—J. Biol. Chem., 114, 475.

[14] Baeyer and Villiger, (1901).—Ber. Chem. Gesselschaft, 34, 738.

———— (1929).—Ibid., 62, 218.

[15] Rieche and Hitz, (1927).—Ibid., 62, 2473.

[16] Guthrie, J. D., (1930).—Jour. Amer. Chem. Soc , 52, 3614.

[17] Bruckman, G. and Zondek, S. G., (1940).—J. Biol. Chem., 135, 24.

[18] Yabusoe, (1925).—Biochem. Zeitschrift, 157, 388.

[19] Kar, B. K. and Banerjee, H. K., (1938-1939).—Trans. Bose Res. Inst., Vol. XIV, 91.

[20] Banerjee, H. K. and Kar, B. K., (1941).—Current Sc., Vol. 10, 289.

[21] Bagster, L. S., (1940).—Chem. Abs., 34, 4174.

XIII.   ON THE ELASTIC SCATTERING OF MESON BY A COULOMB FIELD.   I

By R. C. Majumdar and S. Gupta

Summary

The scattering of meson by a Coulomb field has been calculated for different values of its spin.   The contribution to the scattering from the effects of the finite size of the nucleus and the screening of the nucleus by the atomic electrons are also estimated.   These are preliminary to the calculations of a theory of multiple scattering of mesons which will be given in the second part of the paper.

Introduction

The relativistic theory of the scattering of a charged particle by a Coulomb field was first worked out by Mott[1] from Dirac's theory of electron.   The calculation was later on much simplified by Sauter[2].   The experiments on the scattering of fast electrons by the nuclear field have been carried out by several workers and their results found to agree satisfactorily with Mott's theory.   The relativistic theory of scattering has been further subjected to statistical treatment by Williams[3] in order to develop a theory of multiple scattering of electrons.   Such a theory was, however, first given by Bothe[4] and Wentzel[5] from a classical theory of collisions.   The problem of multiple scattering is important in connection with the experiments performed by Anderson[6], and Blackett and Wilson[7] on the scattering of cosmic ray particles by a metal plate about one centimetre thick placed in the path of the cosmic rays in Wilson chamber.   It was shown by Williams that the scattering under these conditions was mainly multiple and his theory was in fair agreement with the experiments.   But it should be mentioned that the scattering observed by Blackett and Wilson for particles with energy greater than 2×10^8 e.v. are mostly due to mesons, and Williams in his theory used the same relativistic formula for electrons and mesons without any consideration of the spin effect. Williams justifies this by showing that the correction due to the spin of the particle is negligible at small angles for which the experiments on scattering have been performed.   The scattering of meson of spin one obeying Proca's equation has been recently discussed by Laporte[8].   It will be found from the discussion of the present paper that the spin of the meson contributes considerably to the scattering at large angles for even moderate incident energy and at small angles for relatively high energy of the meson.   This consideration therefore requires a revision of Williams' calculations on the multiple scattering

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of meson, which will be taken up in the present paper. As a preliminary to these calculations we propose in the first part of the present paper to calculate the scattering of meson with different spin values by the Coulomb field of the nucleus. The corrections to this scattering due to the effects of the finite size of the nucleus and the screening of the nucleus by the atomic electrons are also worked out. The results of the scattering of meson with different spins are illustrated in the accompanying figures. The theory of the multiple scattering of meson and its application to the experiments on cosmic rays will be discussed in the second part of the paper.

§ 1. THE INTERACTION MATRIX ELEMENTS

We shall first calculate the matrix elements for the transition of meson with different spins in a Coulomb field of the nucleus.

(i) The interaction matrix elements due to the transition of a meson of spin one and obeying Proca's equation from a state $\vec{k}$ to the state $\vec{k}'$ in the static Coulomb field is, in the notation of Heilter [9], given by §

$$H = \frac{ie}{4\pi\hbar c}\int\left[\left\{V\left(\lambda\vec{\Psi}+\frac{1}{c}\vec{\Phi},\ \vec{\Phi}^*-\frac{1}{c\lambda}\vec{\Psi}\right)\right\}-\text{conj. comp.}\right]d\tau\ ..\quad(1)$$

Here V is the electrostatic potential. $\vec{\Phi}$ and $\vec{\Psi}$ are vectors representing the wave functions for the transverse and longitudinal mesons respectively. These can be expanded in plane waves and expressed, after quantising according to Pauli-Weisskopf [10] formalism, as

$$\begin{aligned}
\vec{\Phi} &= i\hbar c\sum_k\sqrt{\frac{2\pi}{E_k}}(a_k-b_k^*)\vec{j}\,e^{i(\vec{k},\vec{r})} \\
\vec{\Psi} &= i\hbar c\sum_k\sqrt{\frac{2\pi}{E_k}}(A_k-B_k^*)\frac{\vec{k}}{k}\,e^{i(\vec{k},\vec{r})} \\
\dot{\vec{\Phi}} &= c\sum_k\sqrt{2\pi E_k}\,(a_k+b_k^*)\vec{j}\,e^{i(\vec{k},\vec{r})} \\
\dot{\vec{\Psi}} &= c\sum_k\sqrt{2\pi E_k}\,(A_k+B_k^*)\frac{\vec{k}}{k}\,e^{i(\vec{k},\vec{r})}
\end{aligned}\qquad(2)$$

where k is the wave vector in the direction of propagation and j the unit vector in the direction of polarisation of the transverse meson. The operators a, a^*, b, b^*, have matrix elements different from zero for transitions in which the number of positive or negative transverse mesons decreases or increases by one. The corresponding capital letters have the same significance for the longitudinal mesons. It should be noticed that the wave vector $\vec{k}$ is connected

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§ *Note.*—ħ denotes the Planck's constant divided by 2π.

with the momentum of the meson in a different way for positive and negative mesons. For positive meson we have $\vec{p} = c\underline{h}\vec{k}$ and for negative meson $\vec{p} = -c\underline{h}\vec{k}$. The introduction of wave number gives the symmetry between positive and negative mesons and our calculations are therefore valid for mesons with both signs.

Further $\qquad E_k = \underline{h}c\sqrt{\overline{k^2+\lambda^2}}, \quad \lambda = \dfrac{Mc}{\underline{h}}$ .. .. .. (3)

With the substitution of (2) the matrix element (1) reduces to

$$H = \sum_k \sum_{k'} V_{\vec{k}\,\vec{k}'}\left[\frac{1}{2\sqrt{E_k E_{k'}}}\left\{(E_k+E_{k'})(A_k A_{k'}^* - B_k^* B_{k'})\left(\vec{n}\,\vec{n}'\right)\right.\right.$$

$$\left.+(E_k+E_{k'})(a_k a_{k'}^* - b_k^* b_{k'})\left(\vec{j}\,\vec{j}'\right)\right\} + \frac{i\lambda\underline{h}c}{2\sqrt{E_k E_{k'}}}\left\{\left(1+\frac{E_k E_{k'}}{\lambda^2\underline{h}^2 c^2}\right)(A_k a_{k'}^* - B_k^* b_{k'})\left(\vec{n}\,\vec{j}'\right)\right.$$

$$\left.\left.-\left(1+\frac{E_k E_{k'}}{\lambda^2\underline{h}^2 c^2}\right)(a_k A_{k'}^* + b_k^* B_{k'})\left(\vec{n}'\,\vec{j}\right)\right\}\right] \qquad .. \qquad .. \quad (4)$$

In the above matrix element we have retained only those terms which are relevant to our present problem. The terms giving rise to simultaneous emission or absorption of two mesons have been omitted. We have further put $\vec{n} = \dfrac{k}{k}, \qquad \vec{n}' = \dfrac{k'}{k}, \quad$ and

$$V_{\vec{k}\,\vec{k}'} \qquad \int V e^{i\left(\vec{k}-\vec{k}',\vec{r}\right)} d\tau \qquad .. \qquad .. \qquad .. \quad (5)$$

We write down now the matrix elements explicitly for the following transition of meson before and after the scattering.

$$H^{\text{long-long}} = \frac{V_{\vec{k}\,\vec{k}'}}{2}\frac{E+E'}{\sqrt{EE'}}\left(\vec{n}\,\vec{n}'\right)$$

$$H^{\text{trans-trans}} = \frac{V_{\vec{k}\,\vec{k}'}}{2}\frac{E+E'}{\sqrt{EE'}}\left(\vec{j}\,\vec{j}'\right)$$

$$\left.\begin{array}{l} \\ \\ \\ \\ \end{array}\right\} .. \qquad .. \quad (6)$$

$$H^{\text{long-trans}} = \frac{i\lambda\underline{h}c}{2}\frac{V_{\vec{k}\,\vec{k}'}}{\sqrt{EE'}}\left(1+\frac{EE'}{\lambda^2\underline{h}^2 c^2}\right)\left(\vec{n}\,\vec{j}'\right)$$

$$H^{\text{trans-long}} = \frac{-i\lambda\underline{h}c}{2}\frac{V_{\vec{k}\,\vec{k}'}}{\sqrt{EE'}}\left(1+\frac{EE'}{\lambda^2\underline{h}^2 c^2}\right)\left(\vec{n}'\,\vec{j}\right)$$

(ii) For meson of spin zero and obeying Pauli-Weisskopf equation the corresponding matrix element is given by

$$H = \frac{V_{\vec{k}\,\vec{k}'}}{2}\frac{E+E'}{\sqrt{EE'}} \quad .. \qquad .. \qquad .. \qquad .. \quad (7)$$

(iii) We can similarly write down the matrix element for meson of spin half and obeying Dirac's equation in the following form.

$$H = V_{\vec{k}\,\vec{k}'}(u^{*'}u) \quad .. \qquad .. \qquad .. \qquad .. \qquad (8)$$

where u and u' are the amplitudes of Dirac's wave equation for the state $\vec{k}$ and $\vec{k}'$.

§ 2.   EVALUATION OF $V_{\vec{k}\,\vec{k}'}$ FOR THE SCREENING AND FINITE SIZE OF THE NUCLEUS.

We now proceed to evaluate the matrix $V_{\vec{k}\,\vec{k}'}$ for the following potential fields of the atom.   In considering this we should remember that the atomic field at large distances greater than $a = a_0 z^{-\frac{1}{3}}$, where a_0 is the radius of first Bohr orbit of Hydrogen atom, is screened by the atomic electrons, whereas for small distances, in the neighbourhood of the nucleus, the electrostatic field largely deviates from the Coulombian field due to the distribution of nuclear charge over the finite volume.   For the field which takes into account of the screening by the atomic electrons we can write.

$$V = \frac{ze^2}{r} e^{-\frac{r}{a}} \quad .. \qquad .. \qquad .. \qquad .. \qquad (9)$$

Substituting this in (5) and integrating we obtain

$$V_{\vec{k}\,\vec{k}'} = \frac{4\pi ze^2 a^2}{1 + \left|\vec{k}-\vec{k}'\right|^2 a^2} = \frac{4\pi ze^2}{\left|\vec{k}-\vec{k}'\right|^2} \frac{1}{1 + \dfrac{1}{\left|\vec{k}-\vec{k}'\right|^2 a^2}} \quad .. \qquad .. \qquad (10)$$

This reduces to the case of pure Coulomb field ($a \to \infty$)

when $\qquad\qquad\qquad .. \qquad \dfrac{4\pi ze^2}{} \qquad\qquad .. \qquad (11)$

The effect of the finite size of the nucleus becomes important for the high energy particles as we are concerned with in cosmic ray experiments.   For, the wavelength of the incident meson being in this case less than the nuclear dimension the interference from the different charge centres in the nucleus should be taken into account which will have a reducing effect on the scattering. Assuming the charge distribution to be uniform and spherical we can approximately take the field near the nucleus as

$$V = \frac{ze^2}{r}\left(1 - e^{-\frac{r}{d}}\right) \quad .. \qquad\qquad\qquad (12)$$

Where d is the nuclear radius and, according to the statistical model of nucleus, is given by $d = r_0 Z^{\frac{1}{3}}$, $r_0 = \dfrac{5}{6}\dfrac{h}{Mc} = 1\cdot60 \times 10^{-13}$ cm., for $M = 200m$. We obtain therefore from (12) and (5)

$$V_{\vec{k}\vec{k}'} = \frac{4\pi z e^2}{|\vec{k}-\vec{k}'|^2\, 1+|\vec{k}-\vec{k}'|^2\, d^2} \qquad \cdots \qquad \cdots \qquad \cdots \quad (13)$$

§3. Calculation of cross-sections

The differential cross-section for the scattering of meson within the solid angle $d\Omega$ is given by

$$d\phi = \frac{1}{4\pi^2 \underline{h} v}\,|\,H\,|^2\, k'^2\,\frac{dk'}{dE_F}\,d\Omega \qquad \cdots \qquad \cdots \qquad \cdots \quad (14)$$

where H is the interaction matrix given above, E_F the total energy in the final state and v the velocity of the incident meson.   For the case of elastic scattering, where $E = E'$, $\left|\vec{k}\right| = \left|\vec{k}'\right|$, the cross-section (14) reduces to

$$d\phi = \left(\frac{M}{2\pi\underline{h}^2}\right)^2 \gamma^2\,|\,H\,|^2\, d\Omega \qquad \cdots \qquad \cdots \qquad \cdots \quad (15)$$

with
$$\gamma = \frac{1}{\sqrt{1 - \dfrac{v^2}{c^2}}} \qquad \cdots \qquad \cdots \qquad \cdots \qquad \cdots \qquad \cdots \quad (16)$$

Now to obtain the scattering of meson of spin one we shall have to average over the initial and sum over the final polarisations of the meson in the expression (15).   Denoting this process by bars we easily obtain the following relations

$$\overline{\left(\vec{n}\,\vec{n}'\right)^2} = \tfrac{1}{3}\cos^2\theta,\ \overline{\left(\vec{j}\,\vec{j}'\right)^2} = \tfrac{1}{3}(1+\cos^2\theta),\ \overline{\left(\vec{n}'\vec{j}\right)^2} = \overline{\left(\vec{n}\,\vec{j}'\right)^2} = \tfrac{1}{3}\sin^2\theta \quad (17)$$

Combining these relations with the matrix elements given by (6) we can express the cross-section (15) for the case of pure Coulomb field (11) as follows

$$d\phi = \left(\frac{e^2 z}{2Mv^2}\right)^2 \frac{1}{\sin^4\frac{\theta}{2}}\frac{1}{\gamma^2}\left\{1+\frac{(\gamma^2-1)^2}{6\gamma^2}\sin^2\theta\right\}d\Omega \qquad \cdots \quad (18)$$

The corresponding cross-section for the scattering of meson of spin zero is at once obtained from (7), (11) and (15) as

$$d\phi = \left(\frac{e^2 z}{2Mv^2}\right)^2 \frac{1}{\sin^4\frac{\theta}{2}}\frac{1}{\gamma^2}\,d\Omega \qquad \cdots \qquad \cdots \qquad \cdots \quad (19)$$

For the case of scattering of meson of spin half we shall have to average over the initial and sum over the final spins of the electrons in the expression (15). Thus we obtain

$$|H|^2 = |V_{\vec{k}\,\vec{k'}}|^2 \left(1 - \frac{\gamma^2 - 1}{\gamma^2} \sin^2 \frac{\theta}{2}\right), \qquad .. \qquad .. \quad (20)$$

whence the cross-section for the case of pure Coulomb field follows immediately from (15 and (11)

$$d\phi = \left(\frac{e^2 z}{2Mv^2}\right)^2 \frac{1}{\sin^4 \frac{\theta}{2}} \frac{1}{\gamma^2} \left\{1 - \frac{\gamma^2 - 1}{\gamma^2} \sin^2 \frac{\theta}{2}\right\} d\Omega .. \qquad .. \quad (21)$$

The effect of the screening, as can be seen from equation (10), is to multiply the scattering cross-section by a factor $\left\{1 + \left(\frac{\lambda}{2a} \Big/ \sin \frac{\theta}{2}\right)^2\right\}^{-2}$, where the wavelength of the incident meson $\lambda = \frac{hc}{p}$. This is equal to unity when $\theta \gg \frac{\lambda}{a}$ and leaves therefore the scattering unaltered. But when $\theta \ll \frac{\lambda}{a}$ the scattering is very much reduced. The consideration of the effect of the nuclear size, on the other hand, requires, as evident from (13), that the scattering cross-section should be multipled by a factor $\left\{1 + \left(\frac{2d}{\lambda} \sin \frac{\theta}{2}\right)^2\right\}^{-2}$. Thus for $\theta \ll \frac{\lambda}{d}$ the scattering is very little affected, whereas for $\theta \gg \frac{\lambda}{d}$ it is greatly reduced. We therefore find that the screening and the nuclear size have got so to say a cut-off effect on the scattering due to pure Coulomb field, restricting it mainly within the angular range lying between $\frac{\lambda}{a}$ and $\frac{\lambda}{d}$. When the wavelength of the incident meson is much less than the nuclear dimension the scattering will be confined to small angles only. The consideration of nuclear size thus imposes a restriction, namely $\Delta p \ll \frac{hc}{d}$, on the transfer of momentum to the nucleus during the collision.

§ 4. DISCUSSION

We find from (21) and (19) that the ratio of the scattering cross-sections of meson of spins half and zero is given by $1 - \frac{\gamma^2 - 1}{\gamma^2} \sin^2 \frac{\theta}{2}$ which is nearly unity for all small angles with which we are generally concerned in our scattering experiments. The spin of the meson obeying Dirac's equation has therefore practically no effect on the scattering at small angles and can be left out of account as done by Williams. But when the meson has spin one and obeys Proca's
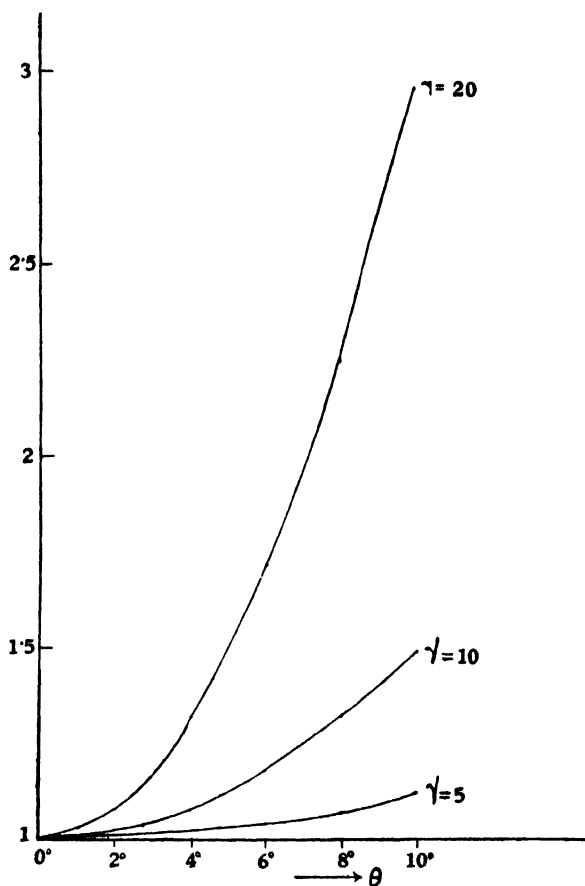
FIG. 1

equation the corresponding ratio of the scattering cross-sections for spins one and zero becomes, according to (18) and (19), $1 + \dfrac{(\gamma^2 - 1)^2}{6\gamma^2} \sin^2 \theta$ which is also the ratio between the cross-sections of mesons of spins one and half at small angles. This ratio is always greater than one and increases with the square of the incident energy of the meson. The characteristic increase of scattering with the incident energy is noteworthy and is due to the transition of meson from the longitudinal to the transverse state and *vice versa*. For comparison we have drawn in the following figures the ratios of the scattering cross-sections of mesons of different spins. In figure 1 we have drawn $1 + \dfrac{(\gamma^2 - 1)^2}{6\gamma^2} \sin^2 \theta$ as ordinate against the scattering angle θ as abscissa up to $10°$ in a magnified scale for the energies of meson 5×10^8 e.v., 10^9 e.v. and 2×10^9 e.v. We find that the spin effect is already appreciable within this region and becomes quite considerable at still higher energies of the meson.

As for example when the energy of the meson is 10^{10} e.v. the scattering for spin one at an angle of 4° is about 9 times the scattering for spin half or zero whereas at an angle of 10° the ratio comes out to be as high as 51. In the figure 2

FIG. 2

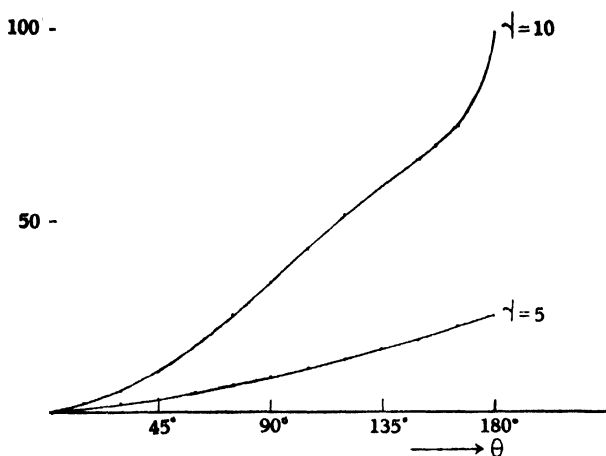we have drawn the ratio of the scattering cross-sections of mesons of spins one and half, i.e. $1+\dfrac{(\gamma^2-1)^2}{6\gamma^2}\sin^2\theta \Big/ 1-\dfrac{\gamma^2-1}{\gamma^2}\sin^2\dfrac{\theta}{2}$ in order to get an idea of the relative contributions of the spins at large angles. It is found that the scattering of meson of spin one is, even at an energy of 10^9 e.v., 10 times the scattering of meson of spin half at an angle of 45° and 100 times at an angle of 180°. The significance of these results in the theory of multiple scattering of meson will be discussed in the second part of the paper.

REFERENCES

1 N. F. Mott.—Proc. Roy. Soc. A., 124, 425, 1929.
2 F. Sauter.—Zs. f. Phys., 86, 818, 1933.
3 E. J. Williams.—Proc. Roy. Soc. A., 169, 539, 1939; Phys. Rev., 58, 292, 1940.
4 W. Bothe.—Zs. f. Phys., 54, 161, 1929.
5 G. Wentzel.—Ann. Phys. Lpz., 69, 335, 1922.
6 C. D. Anderson.—Phys. Rev., 43, 381, 1933.
7 P. M. S. Blackett and J. G. Wilson.—Proc. Roy. Soc. A., 165, 209, 1938.
8 O. Laporte.—Phys. Rev., 54, 905, 1938.
  S. W. Massey and H. C. Corben.—Proc. Camb. Phil. Soc., 35, 463, 1939.
9 W. Heilter.—Proc. Roy. Soc. A., 166, 529, 1938.
10 W. Pauli and V. Weisskopf.—Helv. Phys., 7, 709, 1934.

XIV. PRELIMINARY OBSERVATIONS ON THE CHROMOSOME MORPHOLOGY IN ASIATIC COTTONS WITH SPECIAL REFERENCE TO THEIR PHYLOGENY AND INTER-RELATIONSHIPS *

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I. INTRODUCTION

An intimate knowledge of the inter-relationships of varieties and species in a genus is an essential pre-requisite in any programme of hybridization work. Several investigators have tried to tackle this problem in cottons from various angles. Harland (1936), Hutchinson (1938), Hutchinson and Ghose (1937) and Zaitzev (1928) based their conclusions mainly on the analysis of the external morphology, geographical distribution and the genetic behaviour of the several types. Abraham and Ramanatha Ayyar (1938 and unpublished records) in a survey of the vascular anatomy of the flowers of the wild and cultivated cottons disclosed some new characters in their vascular systems which aided in tracing their relationships. Skovsted (1937) and Webber (1935) on the other hand relied chiefly on the ability of the types to hybridize and the relative fertility of the hybrids together with an analysis of the pairing affinities of the chromosomes. While pairing affinity may indicate chromosome homology to a great extent, it is well known that homologous chromosomes do not always pair. Muntzing (1935) in *Nicotiana digluta* reports failure of pairing between obviously homologous chromosomes. Levitsky and Benetzkaia (1929 and 1931) also find a similar condition in an amphidiploid wheat-rye hybrid. Meurman (1928) reports in *Ribes gordonianum* complete conjugation in certain cells of smaller size lying towards the middle of the anther, while some cells near the periphery show only univalents. He thinks that this difference in conjugation is due to the differences in nutritive conditions. Stowe (1926 and 1927) ascribes the complete failure of pairing observed in *Solanum tuberosum* to higher temperature prevalent there. Rosenburg (quoted by Federley, 1932) finds in *Hieracium lævigatum* and *H. lacerum* that the age of the flower may influence chromosome pairing. Gowen (1928) in *Drosophila* discovered a gene which affects conjugation and prevents crossing over. On the other hand, although homologous chromosomes may fail to pair occasionally, pairing between non-homologous chromosomes

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* Part of paper read at Bombay at the 'Second Conference of Scientific Research Workers on Cotton in India' on the 20th January, 1941.

takes place under exceptional conditions. Longley (1924, 1926) in *Rubus* and *Citrus* ; Gates (1909) in a triploid *Oenothera*, Yarnell (1931) in a triploid *Fagaria* ; McClinlock (1934) in *Zea mays* and Lammerts (1934) in a haploid *Nicotiana* reported or inferred pairing between obviously non-homologous chromosomes or segments.

A critical study of the somatic chromosomes is instructive in various ways. It brings to light not only the numerical variation (polyploidy and aneuploidy) but also indicates the nature of evolution. of the chromosome complements in the various species and allied genera. The size of the chromosomes together with the positions of the spindle attachment regions which determine the arms of the chromosomes, the secondary constrictions and the satellites indicate the chromosome type in a species. Any changes observed in the positions of these lead to the inference of translocation, fusion or fragmentation, according to the changes involved. Studies of the karyotype alterations on these lines in several plant groups such as *Liliaceæ* (Delaunay, 1926), (Sato, 1936), *Crepidineæ* (Babcock *et al.*, 1937), *Allium* (Levan, 1932) and *Cassia* (Jacob, 1940*a*) have furnished useful information regarding their relationships.

Although the somatic chromosomes of several types of cotton have been investigated before, yet little attention was paid by investigators to find out the number of satellited chromosomes in the complement and their relationship to the nucleoli. The usefulness of such a study first came to be recognized when De Mol (1926) pointed out that the number of nucleoli in the cells was a reliable guide to the polyploidy of the plant, diploids having 2 nucleoli, triploids 3 and tetraploids 4. Later Heitz (1931*a* and *b*) showed that the number of nucleoli formed in the telophase nuclei depended upon the number of SAT-chromosomes in the complement. Gates (1938) has shown how the study of the satellites and nucleoli can be brought to bear on the phylogeny of the nucleus and a variation in their number and size correlated with auto or allo polyploidy. These data when correlated with the maximum secondary pairing will form a reliable guide to the basic number of the species or genera. Thus it has been pointed out that three species of *Lactuca* ($2n = 16$) investigated by Babcock *et al.* (1937) are secondary tetraploids of amphidiploid origin, each having 4 SAT-chromosomes in the complement. Again rice which has $n = 12$ chromosomes has been shown to have originated through secondary polyploidy from an ancestral condition in which $n = 5$ (Nandi, 1936). Similarly, *Sesbania speciosa* and *S. Sesban* (Jacob, 1941*a*) have been shown to be secondary tetraploids, evolved from a basic set of 4.

The present approach to the subject, viz., by a critical examination of the somatic chromosomes with special reference to the number and nature of the nucleolar chromosomes, the nucleoli formed at telophase and the attachment of these nucleolar chromosomes to the nucleoli at prophase by the ' Feulgen-Fast green technique ' (Jacob, 1941*a*) though not complete in itself, will, however, form a reliable guide towards a better understanding of the

problem, of inter-relationship, between species. Studies on similar lines in several plant genera such as *Sesbania* (Jacob, 1941*a*), *Brassica* (Sikka, 1940*b*), *Crocus* (Pathak, 1940*b*) and *Oenothera* (Bhaduri, 1940) have furnished interesting results.

II. Previous Work

A detailed critical study of the nucleolar chromosomes in cotton has not been made by any investigator before. The necessity for such a study was felt by Gates (1938) in discussing ' the origin of the cultivated cotton' where he suggested that the two alternative basic numbers, viz., 6 and 7 inferred by Skovsted and Davie respectively 'can be further investigated by a study of the satellites attached to the chromosomes in the various species and the number of nucleoli produced by each'.

Even the results of the karyotype analysis in cottons are contradictory to a certain extent. This is chiefly due to the small size of their nuclei which contains a fairly large number of small chromosomes together with the imperfect techniques employed. Skovsted (1933 and 1934) investigated the somatic chromosomes of *G. stocksii* and *G. arboreum* where he indicates 4 satellited chromosomes. Arutjunova (1936) in an investigation of the somatic chromosomes of *G. herbaceum* found that they could be analyzed and classified into 13 types of which 7 showed secondary differentiations in the forms of satellites, heads and secondary segments. The most detailed study of the size and morphology of the somatic chromosomes in Asiatic cottons is that of Abraham (1940). He investigated the following species, *G. stocksii*, *G. arboreum*, and *G. herbaceum* where he has figured two pairs of satellited chromosomes. But the present investigations on three varieties each of *G. herbaceum* and *G. arboreum* as well as *G. stocksii* showed the presence of only one pair of satellited chromosomes. This is being discussed elsewhere in this paper.

III. Material and Methods

The following species and varieties were investigated :—

1. *G. stocksii* Mast.
2. *G. arboreum* L. var. *typicum* forma *indica* H. & G. (Nadam).
3. Do.   do.   *neglectum* forma *bengalensis* H. & G. (*roseum*).
4. Do.   do.   *cernuum* H. & G. (*cernuum* from Garo Hills).
5. *G. herbaceum* L. var. *africanum* H. & G.
6. Do.   do.   *frutescens* Delile (Uppam, 2919).
7. Do.   do.   *typicum* H. & G. (Russian, 2282).

Root tips were collected from seedlings germinated between moist blotting sheets on bright days between 8 A.M. and 9-30 A.M. and fixed in Levitsky's fluid (10% Formalin—5 parts and 1% chromic acid—4 parts). A pinch of

maltose added to the fixative at the time of fixation was found to give satis-factory spreading of the chromosomes at metaphase. The fixed materials were washed in tepid water from 3 to 4 hours, dehydrated and cleared by the chloroform method. Sections were cut at 15μ thickness and stained by the Feulgen-Fast green technique (Jacob, 1941a).

IV. MORPHOLOGY OF THE SOMATIC CHROMOSOMES

All the above types showed only one pair of satellited chromosomes each. They were found attached to the nucleolus at somatic prophase. In addition, each type had one pair of secondary constricted chromosome attached to the nucleolus at prophase and 4 nucleoli were produced in connection with these 4 nucleolar chromosomes at telophase. The non-nucleolar chromosomes could be grouped into two, those with median constriction and those with sub-median constriction. These varied in number in the various types and could be classified into 5 groups, depending on their lengths and positions of the constrictions; chromosomes of the same lengths and similar constrictions being grouped under one letter. Thus the haploid constitution of the various types are:—

<div align="center">

G. stocksii.

A
B
C_1 C_1.
C_2 C_2.
C_3 C_3.
C_4 C_4.
D_1 D_1 D_1

</div>

where A represents the secondarily constricted; B the satellited, C the medianly constricted and D the sub-medianly constricted chromosomes. All the medianly constricted (C) and sub-medianly constricted (D) chromosomes are graded according to their lengths in the descending order, indicated by numbers (C_1 the longest and C_4 the shortest and D_1 the longest and D_4 the shortest).

G. arboreum var. *typicum.*	*G. arb.* var. *neglectum.*	*G. arb.* var. *cernuum.*
A	A	A
B	B	B
C_1 C_1 C_1	C_1	C_1
C_2 C_2	C_2 C_2 C_2	C_2 C_2
D_1 D_1	C_3 C_3 C_3 C_3	C_3 C_3 C_3
D_2	C_4	C_4 C_4
D_3 D_3 D_3	D_1 D_1	D_1 D_1 D_1

G. *herbaceum* var. *africanum.*	G. *herb.* var. *frutescens.*	G. *herb.* var. *typicum.*
A	A	A
B	B	B
C_1 C_1	C_1 C_1	C_1
D_1 D_1	C_2 C_2 C_2 C_2 C_2	C_2 C_2 C_2 C_2 C_2
D_2 D_2 D_2	C_3 C_3	C_3 C_3 C_3
D_3 D_3 D_3	C_4	D_1
D_4	D_1	D_2

TEXT FIG. I
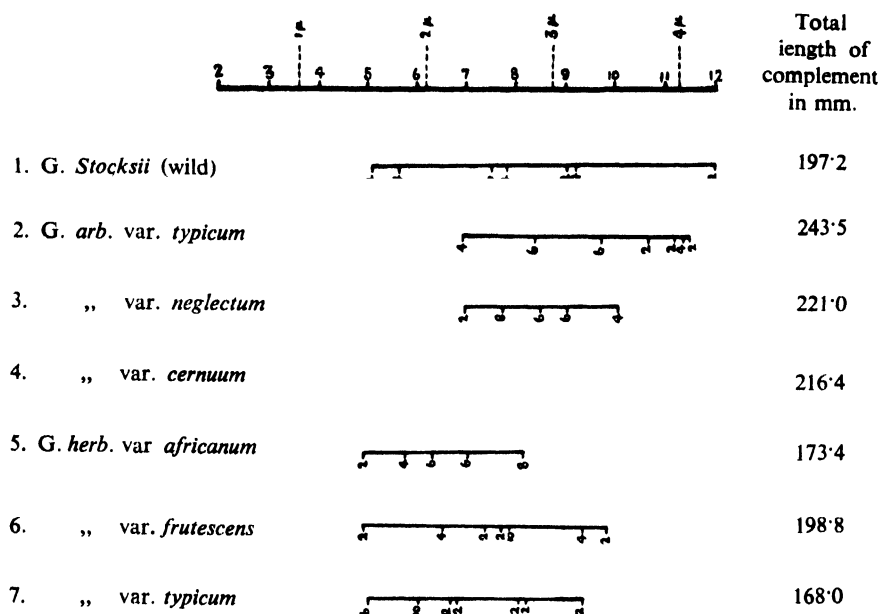
Size frequency and length of Somatic Chromosomes in seven Asiatic Cottons
at a magnification of 2,600 times.

Text Fig. I gives the size frequency and length of the somatic chromosomes in mm. at a magnification of 2,600 times. It will be noticed that the longest chromosomes in *G. stocksii* are much longer than in the other types investigated so far and that there is a wide range between the longest and shortest chromosome pairs. Among the three *arboreum* varieties, the longest chromosomes of *typicum* and *cernuum* and the shortest chromosomes of *neglectum* and *cernuum* are nearly equal. The difference between the longest and the shortest chromosome pairs is greatest in *cernuum* and least in *neglectum*. But the total length of all the chromosomes is greatest in *typicum* and least in *cernuum*. Among the three *herbaceum* types, the shortest chromosomes of all are nearly equal, while *frutescens* has the longest chromosomes and *africanum* the shortest. The total length of all the chromosomes is greatest in *frutescens*.

Plate XIX, figs. 5–11 shows the nucleolar chromosomes in the types examined so far. The satellited as well as secondarily constricted chromosomes of *G. stocksii* (fig. 5) appear to be the longest. Among the *arboreum* varieties, *typicum* (fig. 6) appears to be the longest with a very long filament connecting the satellite to the body of the chromosomes. The shortest satellited chromosomes are in *cernuum* (fig. 8). Among the three *herbaceum* types, *frutescens* (fig. 10) has the longest nucleolar chromosomes. The secondarily constricted chromosomes in *africanum* (fig. 9) and *typicum* (fig. 11) appear to be morphologically similar in that they have their longest segment in the middle. Again, the secondarily constricted chromosomes in *frutescens* (fig. 10) appear to be morphologically similar to that of *G. arboreum* var. *typicum* (fig. 6) and *neglectum* (fig. 7) in that they have their longest segment towards the end.

V. DISCUSSION

Satellited and secondary constricted chromosomes.—Although the presence of satellites in the chromosome complement of plants had been demonstrated as early as 1912 by Navashin in *Galtonia*, its true significance came to be realized only much later. The true relationship between satellites and nucleoli was first recognized by Heitz (1931*a* and *b*) when he inferred that all plants might have satellited chromosomes which gave rise to nucleoli in telophase. In a subsequent paper he came to the conclusion that secondarily constricted chromosomes might also produce nucleoli. Morphologically the satellited and secondarily constricted chromosomes are similar in that they have the primary as well as secondary constrictions, but with this difference that the connection at the ' secondary constriction' is long and drawn out in the former while in the latter they are much shorter with the result that the distal segment is close to the body of the chromosome. It has been shown elsewhere that all the previous workers reported the presence of two pairs of satellited chromosomes in the somatic complement of the Asiatic cottons. Taking into consideration the results of the present investigation, it necessarily means that a pair of secondarily constricted chromosomes were taken to be satellited. Such a mistake is quite possible especially in those cases where the distal segment is small. This may ' swell up' and appear as a rounded body with imperfect fixations or with those fixatives containing acetic acid (Navashin). An examination of the illustrations of the somatic chromosomes of Skovsted (1933 and 1935) clearly show that only one pair in each diploid complement is normally satellited where the connecting filament is rather long. In the other ' satellited pair' the so-called satellite is so close to the body of the chromosome to suggest the possibility of it being a small distal segment. This is all the more likely since Skovsted used a fixative containing acetic acid (Navashin) which will ' swell up' the chromosomes. In this connection it may be mentioned that Camara (1940) in a tetraploid wheat states that a normally secondarily constricted pair is often mistaken to be a satellited pair in several plates. This discrepancy can easily be cleared up by an examination of the somatic prophase. Fig. 3 shows the somatic prophase in *G. arboreum* var. *typicum* (H. & G.) where two chromosomes are attached to the nucleolus by

means of satellites, while in the other two, the attachments to the nucleolus appear to be sub-median—corresponding to the secondary constriction (figs. 1 and 2).

Origin of the Asiatic diploids.—From an analysis of the secondary associations of the bivalents at metaphase I, Davie concluded 7 to be the basic number of the genus *Gossypium*.   This was further substantiated by the occurrence of 7 or its higher multiples in such genera as Lavatera, Malva, Malvastrum, Kitaibelia and Pavonia.   Skovsted (1937) also showed a few cases where the 13 chromosome pairs arranged themselves in six pairs with one single also indicating 7 to be the basic number.   He has, however, found in 5 cells a group of 3 bodies in addition to various pairs indicating that the previous chromosome number was 6.   It is supported by the finding that the genera Gossypioides and Kokia, allied to *Gossypium* have $2n = 24$.   Gates (1938) when reviewing the previous work on the origin of the cultivated cotton concludes that 7 was the primary basic number from which the haploid number 13 of the present-day cottons arose, 6 being a transitional number.   This seems to be highly probable especially as 7 groups of chromosomes are in all the types examined.   The whole question will be dealt with in a subsequent paper.

The importance of the study of the nucleolar chromosomes in evaluating the phylogeny of species in a genus was first recognized by Gates and his school of workers, which is dealt with elsewhere in this paper.   Thus on the assumption that a primary diploid will have only 2 nucleolar chromosomes which produces only 2 nucleoli at telophase, *G. arboreum* var. *typicum* with its 4 nucleolar chromosomes (figs. 1 and 2) at metaphase which produces 4 nucleoli at telophase (fig. 4) cannot be taken as a true diploid.   But it must be stated that the two pairs of nucleolar chromosomes are dissimilar, one pair being satellited and the other secondarily constricted.   The presence of heteromorphic nucleolar chromosomes suggest that hybridization has taken place in the evolution of the genus.   The following tentative hypothesis is suggested regarding the origin and evolution of the Asiatic diploids:—

TEXT FIG. II
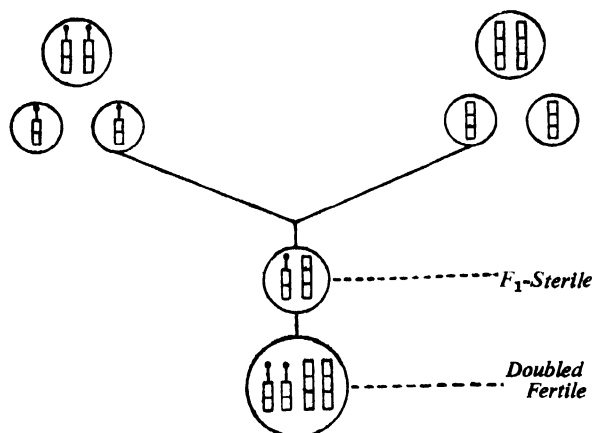
Only the nucleolar chromosomes are represented in the above illustration. On this basis the present-day Asiatic cottons should be considered as secondary allotetraploids with the constitution $4b-2$ where b (the basic number) $= 7$. It may be mentioned in this connection that the American diploids appear to be secondary autotetraploids in that they have two pairs of morphologically similar satellited chromosomes.

Inter-relationships.—It is proposed to discuss here only those points which arise from a study of the nucleolar chromosomes in the 7 types examined. Figs. 5–11 (Plate XIX) illustrate the nucleolar chromosomes in all the above types. It will be seen that the nucleolar chromosomes of *G. arboreum* types are longer than those in the *G. herbaceum* types. *G. stocksii* the wild type appears to have the longest nucleolar chromosomes. Now, in the course of evolution, structural changes such as fragmentation, inversion, deletion and translocation are taking place in the plant. Many of these structural changes result in the progressive shortening of the chromosomes on the principle that only broken ends unite and any acentric segment tends to get lost in the succeeding division stages. In *G. arboreum* var. *typicum*, the satellite filament is very long which is mechanically unsound with the result that a shortening of the satellite filament tends to take place. Thus the *neglectum* may represent a later evolutionary stage and *cernuum* a still later stage. Again the secondarily constricted chromosomes of *neglectum* and *typicum* have their longest segment at one end and hence they appear to be more closely related. In the *G. herbaceum* types, *typicum* has the shortest nucleolar chromosomes and hence appear to be the most highly evolved type. The secondary constricted chromosomes of *G. herbaceum* var. *frutescens* appear to be similar to those in *G. arboreum* var. *neglectum* and var. *typicum* in that they have the longest segment at one end.

It may be mentioned in this connection that Abraham and Ramanatha Ayyar (1938 and unpublished records) in a study of the evolutionary trends and inter-relationships in Asiatic cottons from an analysis of the floral vascular patterns and their inheritance have also arrived at the same conclusions. Hutchinson (1938a) and Hutchinson and Ghose (1937) have also arrived at nearly the same conclusions.

It may now be considered as to how these studies may help in the solution of certain problems facing those engaged in the improvement of cotton. Choice of parents in hybridization work is a problem that has worried many a breeder. It is well known to cotton workers that best results in evolving hybrid strains have been achieved in crosses between types regarded genetically as being members of the same species. Interspecific crosses are often reported to have failed in yielding desirable stable types from an economic standpoint. As an example Harland's (1936) hybridization work between *G. hirsutum* and *G. barbadense* may be cited. He showed that although they cross freely and produce vigorous F_1 plants, they broke down into a range of unproductive and often unbalanced types. The same has been the experience of Hutchinson (1940) and Ramanatha Ayyar in crosses between *G. arboreum* and *G.*

herbaceum. These may have been mainly due to the difference in chromosome constitution of the parents. It is felt that this problem can be tackled successfully to a great extent if a correct idea of the chromosome morphology of the parents is obtained before starting any hybridization programme. Till recently chromosome homology between types has been gauged by the pairing affinities of the chromosomes at meiosis. As already shown elsewhere in this paper, the above phenomenon is in many instances misleading. So the best method of arriving at a correct understanding of the chromosome constitution appears to be from a detailed critical study of the somatic chromosomes with special reference to the nucleolar chromosomes. It is shown earlier, that considerable variations in chromosome constitution exists even between varieties of the same species. In such cases inter-varietal hybridization may not always give stable types from an economic point of view. On the other hand even distant types with morphologically similar chromosomes may result in desirable economic types when hybridized.

VI. SUMMARY

A detailed critical study of the somatic chromosomes with special reference to the nucleolar chromosomes was made in 7 types of Asiatic cottons with a view to trace their phylogeny and inter-relationships.

One pair each, of satellited and secondarily constricted chromosomes were found in all the types. These produced 4 nucleoli at telophase and were found attached to the nucleolus at prophase.

On the basis of length and morphology 7 groups of somatic chromosomes could be identified in all the types.

Evidences from the somatic complements suggest that Asiatic cottons are secondary allotetraploids with the constitution $4b-2$.

From a study of the nucleolar chromosomes, the evolutionary trend in *G. arboreum* varieties could be inferred. Thus *typicum* represents an early stage, *neglectum* a later and *cernuum* a still later stage in the evolution of that species. Among the *G. herbaceum* types, *typicum* appears to be more highly evolved than *frutescens*.

The usefulness of such a study in hybridization work is discussed.

In conclusion, I wish to express my sincere thanks to Rao Bahadur V. Ramanatha Ayyar, Cotton Specialist to the Government of Madras, for giving me every facility in his laboratory to conduct these investigations and to Dr. D. M. Bose, Director of this Institute, for facilities offered to continue these investigations and permission to publish this paper in the *Transactions* of this Institute.

LITERATURE CITED

1. Abraham, P., 1940*b*   .. In. J. Agric. Sci., 10, 300.
2. Abraham, P. and Ramanatha   Proc. 1st Conf. Sci. Res , Workers on cotton in India,
   Ayyar, V., 1938.   I.C.C.C., Bombay.
3. Arutjunova, 1936 ..   .. C. R. Acad. Sci., 3, 37.
15B

4. Babcock, E. B., Stebbins, G. L., Cytologia, Fujii Jubilee, Vol. 188.
and Jenkins, J. A., 1937.
5. Bhaduri, P. N., 1940 · .. Proc. Roy. Soc. Series, B, 128, 353.
6. Camara, A. el. Azevedo Cout- Agronomia Lusitana, 3, 268.
inho, 1939.
7. Delaunay, L., 1926 .. Zeitscher. Zellf. mikr. Anat., 4, 338.
8. Gates, R. R., 1909 .. .. Bot. Gaz., 48, 179.
9. ,, 1938a .. J. Roy. Micr. Soc., 58, 97.
10. ,, 1938b .. E.C.G.R., 15, 1.
11. Gowen, G. W., 1928 .. Science, 68, 211.
12. Harland, S. G., 1936 .. Biol. Rev., 2, 1.
13. Heitz. E., 1931a .. .. Planta, 12, 774.
14. ,, 1931b .. .. Planta, 15, 495.
15. Hutchinson, J. B., 1938a .. Proc. 1st Conf. Sci. Res., Workers on cotton in India,
I.C.C.C., Bombay, 347.
16. ,, 1940 .. J. Genet, 40, 271.
17. ,, and Ghose, Ind. J. Agri. Sci., 7, 233.
R. L. M., 1937.
18. Jacob, K. T., 1940a .. Ann. Bot. N. S., 4, 201.
19. ,, 1941a .. Biblio. Genet., 13, Pt. 3.
20. Lammerts, W. E., 1934 .. Cytologia, 6, 38.
21. Levan, A., 1932 .. .. Hereditas, 16, 257.
22. Levitsky, G. A., Benetzkaia, Proc. U.S.S.R. Con. in Genet., 2, 345.
G. K., 1929.
23. Levitsky, G. A., Benetzkaia, 1931 Bull. Appl. Bot. Genet. and Pl. Breed, 27,
G. K., 1929. 241.
24. Longley, A. E., 1924 .. Amer. J. Bot., 2, 249.
25. ,, 1926 .. J. Washington Acad. Sci., 16, 543.
26. McClintock, B., 1934 .. Zeits. f. Zellf. u. mikr. Anat., 21, 294.
27. Meurman, O., 1928 .. Hereditas, 11, 289.
28. Mol de W. E., 1926 .. La Cellule, 38, 7.
29. Muntzing, A., 1935 .. Hereditas, 20, 251.
30. Nandi, H. K., 1936 .. J. Genet., 33, 315.
31. Narashin, S., 1912 .. .. Bull. Acad. Imp. Sci. St. Petersburgh, 6, 575.
32. Pathak, G. N., 1940 C. . Ann. Bot. N. S., 4, 227.
33. Sato, D., 1936 .. .. Cytologia, 7, 521.
34. Sikka, S. M., 1940 C. .. J. Genet., 40, 441.
35. Skovsted, A., 1933 .. .. Ann. Bot., 47, 227.
36. ,, 1934 .. .. J. Genet., 28, 407.
37. ,, 1935a .. .. J. Genet., 30, 447.
38. ,, 1935b .. .. J. Genet., 31, 264.
39. ,, 1937 .. .. J. Genet., 34, 97.
40. Stowe, I., 1926 .. .. Proc. Imp. Acad., 2, 426.
41. Webber, J. M., 1935 .. J. Agri. Res., 51, 1047.
42. Yarnell, S. M., 1931 .. Genetics, 16, 455.
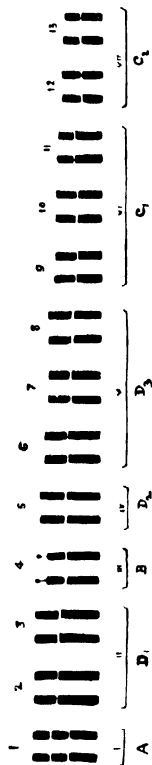43. Zaitzev, G. S., 1928 .. Bull. Appl. Bot. and Pl. Breed., 18, 1.